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TOXICITY IN THE AQUATIC ENVIRONMENT

Edward F. Eldridge

Public Health Service,
Portland, Oregon

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PROCEEDINGS

of the

TENTH PACIFIC NORTHWEST SYMPOSIUM

ON

WATER POLLUTION RESEARCH

TOXICITY IN THE AQUATIC ENVIRONMENT

Assembled by

Edward F. Eldridge

Research & Technical Consultation
Project

U. S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE

Public Health Service

Region IX

Portland, Oregon

November 1961

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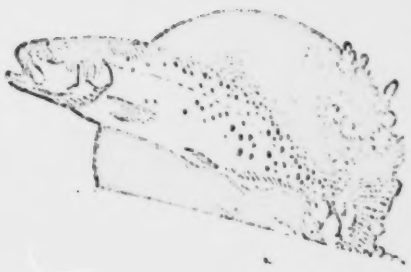

FOREWORD

The Public Health Service in May of 1957 established an activity in the Portland office known as the Research and Technical Consultation Project. The objectives of this project are to provide a closer contact and better service to those persons engaged in research in the water pollution and related fields and to stimulate the interest of others in this field of study.

As a part of this activity, a series of symposia have been held where an opportunity is provided for an informal discussion of subjects relating to water pollution. To date, ten symposiums have been held in Portland covering the following subjects:

1. Research Relating to Problems of Water Pollution in the Northwest
2. Financing Water Pollution Research
3. The Slime (Sphaerotilus) Problem
4. Short-term Bio-Assay
5. Siltation - Its Sources and Effects on the Aquatic Environment
6. Oceanography and Related Estuarial Water Problems of the Northwest
7. Status of Knowledge of Watershed Problems of the Northwest
8. Radioactive Waste Problems in the Pacific Northwest

9. Research in Water Pollution and Other Environmental Health Fields

10. Toxicity in the Aquatic Environment

Over a hundred persons representing universities, colleges, regulatory agencies, and State and Federal departments met in Portland on November 14 for a discussion on the "Toxicity in the Aquatic Environment." Attendance-wise this was the largest symposium to-date indicating a considerable interest in this subject. This report contains the proceedings of this meeting.

TENTH PACIFIC NORTHWEST RESEARCH SYMPOSIUM

SUBJECT: TOXICITY IN THE AQUATIC ENVIRONMENT

DATE: November 14, 1961

PLACE: Hearing Room, Interstate Commerce Commission
410 S. W. Tenth Avenue, Portland, Oregon

AGENDA

A.M.

9:00 - 9:10 — I - Foreword and Introductions - E. F. Eldridge

9:10 - 9:50 — II - Introduction to Toxicity Research -- Scope and Significance of Problem - Dr. C. M. Tarzwell, Chief, Aquatic Biology, Robert A. Taft Sanitary Engineering Center.

III - Pesticides,

9:50 - 10:30 — 1. Public Health Service Studies - Dr. H. P. Nicholson, Chief, Pesticide Pollution Studies, Public Health Service, Atlanta, Georgia.

10:30 - 11:00 — 2. Toxicity of Insecticides to Fish in the Northwest - Dr. Max Katz, Research Associate Professor, College of Fisheries, University of Washington.

11:00 - 11:30 — IV - Toxicity of Metals and Their Detection - Dr. W. Allan Moore, Chief of Laboratory, Public Health Service, Portland, Oregon.

11:30 - 12:00 — V - Pulp Mill Wastes vs. Oysters - Dr. R. E. Dimick, Professor, Fish and Game Management, Oregon State University.

P.M.

1:15 - 1:45 — VI - Process Wastes from Atomic Energy Operations - Mr. P. A. Olson, Biology Operation Section, General Electric Company, Richland, Washington.

1:45 - 2:15 VII - Toxic Effects of Organic Deposits - Mr. Charles Ziebell, Biological Analyst, Washington Pollution Control Commission.

2:15 - 2:45 VIII - Toxicity vs. Agricultural Plants and Soils - Dr. C. D. Moodie, Professor of Soils, Washington State University.

2:45 - 3:15 IX - The Shellfish Toxin Problem -- Effect on Humans - Mr. John Girard, Sanitarian, Washington Department of Health.

3:15 - 3:45 X - Toxicity Bioassays vs. Chemical Methods - Dr. Peter Doudoroff, Fisheries Research, Public Health Service, Oregon State University.

3:45 - 4:15 XI - Bioassay -- The Bi-Valve Larvae Tool - Mr. Charles E. Woelke, Fisheries Biologist, Washington Department of Fisheries.

4:15 - 4:45 XII - Bioassay Studies at Nanaimo, British Columbia - Dr. D. F. Alderdice, Fisheries Research Board of Canada, Biological Station, Nanaimo.

TOXICITY IN THE AQUATIC ENVIRONMENT

INTRODUCTORY REMARKS

E. F. Eldridge*

Life on planet "Earth" is becoming more and more complex. There is some question as to whether Communism and the atomic bomb are our major hazards to life. It may well be that our own uncontrolled disturbance of natural life cycles will be our undoing in the final analysis. This is an awesome picture and may be overemphasized, but it is surely one which should be given serious consideration.

In recent years a great deal of effort has been placed on the production of chemicals foreign to those naturally present in our environment. Many of these are designed to destroy some form of life. These toxic chemicals are certainly not specific in their action. If they are toxic to undesirable forms, they may well be equally toxic to desirable ones. Even the destruction of the so-called "pests" may have an effect on the environment.

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This destruction of life may or may not be desirable, but one thing is certain, it must be subject to control. In order to control the effects of these substances, it is necessary that we know more about them.

The major carrier of such substances is "water." Our objective today is to examine the problems of toxicity in this media and to delineate areas of further study.

INTRODUCTION TO TOXICITY RESEARCH--SCOPE AND SIGNIFICANCE OF PROBLEM

C. M. Tarzwell*

(Note: Dr. Tarzwell's paper was not available in time to be included in these Proceedings. In lieu of this paper, the following abstracts of his statements as taken from the recording of the symposium have been substituted.)

What is a favorable environment? What is the importance for the continued survival and well-being of man? What value should be placed upon a favorable environment and further, what efforts and costs are justified in maintaining this environment? These are important questions and questions which have to be faced in this age of technology.

Men, as well as the aquatic life of our fresh and marine waters, are products of their environment. Through the process of development they became adapted to the physical and chemical conditions which were prevalent at that time. Through the course of time, these environmental conditions have become requirements. These are the things that are required for the survival, growth, reproduction, and general well-being of life. That is the reason why organisms that have evolved in different areas have different environmental requirements.

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Man has learned to change his immediate environment to a certain extent. He has his houses, clothes, and central heating systems with their smells and gases. He has inside toilets, plumbing and sewage system, which takes the waste away from homes into our streams and dumps below town. In these ways, man is controlling his environment.

This has lead him to be somewhat brash in his approach to the problem of the total environment. In his pursuit for wealth and power and the better way of life, he has forged ahead without regard to the effects of the products and by-products of these actions on the total environment. If any of the earth's resources may be said to have some primary importance to continued existence, they are air, soil, and water. If to these is added sunlight, these four constitute the basic requirements for all life on this earth. These are the basic resources.

In this age it seems that man has forgotten his origin and is blind to the real environmental requirements--the conditions under which he developed. Consequently, these resources have become the victims of the modern indifference.

The outstanding problem of the time has become the contamination of the total environment, with substances of incredible potential for harm. Substances that not only exist in the air, and persist in the soil and water, but accumulate in fishes, plants, and animals and

even penetrate germ cells or alter the factors of heredity. Ever since the chemist has manufactured substances which nature never invented, problems have become increasingly complex and the hazards greater. Man only recently began to perceive the potential of these materials for harm. Only recently has he developed an awareness of the consequence of their manufacture and their widespread use and of the waste products resulting from their manufacture. These materials are well known--the petrochemicals, the synthetic organics, the pesticides, the herbicides, the insecticides, the detergents, and other synthetic materials. Many of these compounds are not broken down to bacterial action and their accumulation adds to the problems. They often defy detection or identification by methods in ordinary use in our water treatment plants and other laboratories.

They are unreasonably stable in many instances and conventional methods of waste treatment do not remove them. Once introduced into natural waters, they are a continuing threat not only to the aquatic life, but to man and animals which may wish to drink the water or to use the aquatic animals as food. The use of these materials is widely dispersed and quantities are increasing. For instance, about 637 million pounds of pesticides and 500 million pounds of detergents were manufactured in

1960. These substances are appearing in our surface waters in appreciable quantities and they have also been found in the ground water.

An example is the Colorado experience where the wastes were discharged into the ground and how the effects are creeping over large areas destroying the vegetation and poisoning animals.

This is the problem that man is facing today. Now what is he going to do about it? In approaching any problem, the first requirement is to define terms in order that everyone is talking about the same thing. There are several that are very often used loosely. "Pollution" is one of these. "Pollution" is the addition of any material or any change in water quality or condition which interferes with, lessens or destroys the beneficial use of a water. If there is not an interference with water use, there is not pollution. Therefore, when a person speaks of pollution in general, he very often uses the term loosely.

Another term which should be defined is "toxicity." What is "toxicity"? What determines "toxicity"? Many of the food elements needed for growth, such as sulphur, potassium, calcium, iron, magnesium, and iodine, can become toxic if they are present in large enough quantities or are not buffered. The trace elements especially such as silicone zinc, copper, manganese, molybdenum and boron, can become quite toxic if they are in large enough amounts. However, these

elements also are required for good growth. It is the amount that is of importance. Therefore, a requirement such as "nothing toxic shall be added to a water," is not adequate or reasonable. We need to specify the amount that can be added without causing unsatisfactory conditions.

Bio-assays are used for measuring toxicity, especially for acute toxic effects. They also can be used to show long-term effects through continuous exposure. However, for the long-term effects some physiological tests will eventually have to be used. These will involve the effects on growth, activity, and certain other physiological functions.

Now what are the sources, the nature, and extent of our pollutants? How do they effect the aquatic environment and what are the considerations which should be taken into consideration in approaching the problem? The source of toxicants are the wastes. They may be present in municipal wastes, industrial wastes, or agricultural wastes.

Agriculture should be considered to be an industry with certain wastes. Crops, gardens and forests are treated with materials that have very high toxicity, and very persistent. They reach surface and ground water by runoff or seepage.

In the handling of toxic wastes, consideration must be given to the source. If they originate at a point such as a sewer

outfall, they may be amenable to treatment. There are those that come from land and may be contributed from an entire watershed. These cannot be collected and treated. This means, therefore, that in meeting these problems we must control the material at the source of use. This, of course, applies to silt, fertilizers, and radioactive material, as well as to pesticides and other toxic substances used on vegetation and soils. One exception is the removal of these materials from domestic and industrial water supplies by water treatment.

Among the factors to be considered in a study of toxicity are precipitation, amount and rate of runoff, the season of the year, temperature, the dilution available and, above all, the quality of the receiving water. This latter, of course, is determined by the geology of the area, the type of soil, the vegetation, etc. The quality of the receiving water can be all important in determining the toxicity of materials that was added to it.

Next, it must be recognized that there are great variations in the toxicity of certain materials. For instance, it is found that the organic phosphorus compounds vary greatly in the toxicity to different species of organisms. The greatest variation experienced is that of Delnab, which is 940 times more toxic to bluegills than it is to the goldfish. This may be an extreme range, but among the

organic phosphorus compounds we find wide ranges, such as malathion is about 500 times more toxic to salmon than it is to fathead minnows, and about 250 times more toxic to bluegills. Also, there are differences in tolerance between fish and the aquatic organisms. Endrin is very toxic to fish, whereas some other materials are more toxic to some aquatic insects than they are to fish. Toxicity also varies in the different age groups, and with activity. These variations must be taken into consideration. The most sensitive forms must be protected.

Some substances are acutely toxic, whereas some have an accumulative effect. The time of exposure is another variable.

It is also necessary to consider sub-lethal effects. The species can be eliminated even if the fish or an aquatic organism is not killed. The organism may be so weakened physiologically that its predators and other organisms will overcome it and the species may eventually disappear. All aquatic life is in competition. If the ability of an organism to meet its environment and to compete with other organisms is reduced, it can be effectively wiped out as if it were killed.

This problem of toxicity has not been met effectively to date. There is a lack of knowledge of the amount of each toxic substance which is allowable. The levels of concentrations, both upper and lower, which are required for life must be determined.

The levels of concentration which are acutely toxic and those which are toxic under conditions of continued exposure must be known. To insure growth, survival, reproduction, general well-being, and the production of fish as a crop requires that the most favorable levels of concentration in which to get the best production be maintained.

This is not an easy task and will require research and investigation by many organizations and groups. It will require coordination of effort and the exchange of information. It must be done if the problems present and future are to be met.

In short, water quality criteria is needed--that defines the allowable amounts of certain materials that may be present in the stream. Without this knowledge, pollution cannot be effectively and efficiently detected and evaluated. Neither can a determination be made of the amount and nature of waste treatment required. The quality of water required for each beneficial use must be known. This knowledge is lacking in many areas and especially in the area of toxicity.

It is necessary, therefore, that serious consideration be given to the contamination of the environment. Water is only one source of exposure--there is air, food, etc. The materials from manufacturing processes and their by-products and the products of advanced technology which are leading to what is considered a better way of life,

may in reality be the evil products which can adversely affect the total environment. "Toxicity," therefore, is now a problem which concerns all.

DISCUSSION

STATEMENT: I would like to give several examples of the effect of toxicity. Arsenic is used in herbicides, for insect control, and in the South, in cotton and tobacco fields (Recently new organic insecticides have taken its place in the tobacco field application). From 1932 to 1952 the arsenic in tobacco continuously increased--as much as 300 to 600 per cent. The use of arsenic, therefore, is widespread.

Arsenic was found in a water supply in Argentina. Cancer was very widespread. When the arsenic was removed cancer decreased.

Arsenic was used in the vineyards of Germany for about 20 years (1920 to 1940). Records now show that workers have cancer of the skin, liver, etc. This is a long time effect.

Effects that were not suspected are now turning up. We do not know the effects of such substances in water where they are present for long periods.

Q. Is the Robert A. Taft Sanitary Engineering Center actively working on water quality criteria?

A. We are putting all our eggs in one basket and that is the basket. Yes, we are doing all we can with the funds available. We are now going ahead with physiological studies. We are trying to determine sub-lethal effects. We feel that the physiological studies will be of outstanding importance. It is hoped that these studies will disclose the required water quality criteria for those substances investigated. They will not, of course, specify the criteria to be used in enforcement activities. These must be established by regulatory agencies using the study as a base.

Q. Do you feel that the research in this particular phase of the pollution problem can keep ahead of the development of new chemicals?

A. It hasn't. We need much more research in the biological phase and we might as well face up to it.

Q. Who in your opinion should do this research?

A. Actually, the industry should do this research before the chemical is marketed. I realize that this will cost the industry money and may interfere with the production of new chemicals. Before these chemicals are spread in the environment, we should learn much more about their effects.

DDT, for instance, and some of the insecticides are known to effect the hormones and other physiological functions of humans

and animals. These chemicals are having implications which you never dreamed of. The problem is a product of our civilization and we must face it. Otherwise, we may destroy the very environment in which we originated.

Q. Is there any estimate as to how much we would have to increase our research in order to catch up with the developments to date?

A. Any statement in this regard would be purely a guess, since it is impossible to estimate the time involved in doing research. We cannot just study the simple effects, we have to study the multiple effects in the environment and when we get into that it will take a long time, many people, and considerable money. There is plenty of work for all of us--those that work in the universities, in the states, in the various fisheries departments and in all other interested agencies. It is important, also, that we get together and coordinate as much as possible our efforts in order to avoid duplication. It will take millions of dollars in research in order to protect our aquatic life from the effects of these toxicant materials.

STATEMENT: More biologists working in the water pollution are required, if this research is be done adequately. There are few

universities or colleges in the country making any effort to train biologists in water pollution research. One of the outstanding schools where such training is available is Oregon State University.

Q. In the past there seems to have been money made available in the form of grants for medical and engineering research. Is it now possible that these grants will be more directed toward the biological field?

A. The Public Health Service through the Research and Training Grants Branch now has training and research funds for any person in any school and in any department of those schools, including biology, who will work on water supply and water pollution research. The funds were recently made available by the Congress. The funds are available and we are presently in the process of determining how best to use the money.

PESTICIDE POLLUTION STUDIES IN THE SOUTHEASTERN STATES

H. P. Nicholson*

Technological advances during the past twenty years have resulted in the development of new types of pesticides and vastly expanded use. United States production now exceeds 300,000 tons annually of about 200 basic compounds (1,2). It has been estimated that pesticides are applied annually to over 100,000,000 acres in the United States (2). This usage has generated special concern among those persons and agencies responsible for water pollution control and conservation because

1. Pesticides are poisons by nature even though their destructive qualities are valued when properly controlled.

2. Their effectiveness often demands widespread application to land, forest and water where ultimate disposition cannot always be pre-determined.

3. Destructive side effects in the form of unwanted deaths of aquatic and upland animals, and other incidents, have attracted special attention and marshalled public opinion.

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4. Their development has been so rapid that evaluation of side effects has not kept pace.

Dr. Mark D. Hollis, Assistant Surgeon General and Chief Sanitary Engineer of the United States Public Health Service, warned in 1959, "The treatment of large acreages with weedicides, insecticides, and (other) pesticides results in contamination of water supplies, both surface and ground waters, through percolation and runoff" (3). He pointed out the need to learn more about behavior of chemical exotics in streams, their relations to maximum water reuse, and effects singularly and in combination on aquatic life.

Certain pesticides have occurred in our waters. Middleton and Lichtenburg (4) reported detection of DDT in April, May, and June 1957 in the Mississippi River at Quincy, Illinois, and at New Orleans; in the Missouri River at Kansas City, Kansas; and in the Columbia River at Bonneville, Oregon. Previously it was reported from Lake St. Clair and the Detroit River. They also reported aldrin in a single sample collected at Pullman, Washington, from the Snake River. Sources of these insecticides and circumstances connected with their appearance were not determined.

Herbicides also have been a source of trouble. Cottam (2) reported a brief industrial discharge of 2,4-D in 1945 in California that penetrated the underground aquifers and contaminated a city

water supply for a period of four or five years. He also discussed an instance where 2,4-D apparently was generated from a combination of industrial wastes lagooned near Henderson, Colorado. First noticed in 1951 when crop damage resulted from use of well water for irrigation, some 60 square miles of ground water were at one time said to be contaminated.

More recently the Basic Data Branch of the Public Health Service's Division of Water Supply and Pollution Control assembled data on pollution caused fish kills that occurred in 1960 throughout the United States (5). These data show that 81 of 305, or 27 percent of all fish kills reported, were attributed to agricultural poisons.

Stimulated by a growing awareness of the potentialities for water quality degradation and loss of water resources associated with large scale production and use of pesticides, the Public Health Service began studies in 1959 in the Southeastern States to evaluate the occurrence of pesticides in both surface and ground waters. It was desired to learn if use of these biologically active chemicals was creating more than sporadic water pollution readily detectable by fish kills, crop damage, or the creation of undesirable taste. We wished to know, in brief, how general was the occurrence of pesticides in surface and ground waters, what were the less obvious effects

on organisms living in the aquatic environment, and what factors relate to the presence or absence of selected pesticides in water? These questions have many ramifications involving multiple disciplines. The pesticide pollution studies staff in Region IV includes persons trained in chemistry, entomology, sanitary engineering, limnology, agricultural engineering, and microbiology. As the need arises we borrow the assistance of persons trained in other disciplines from sister agencies such as Geological Survey, Fish and Wildlife Service, Tennessee Valley Authority, and various State Agencies.1/

The first of our studies was begun in June 1959 and still continues in a 400 square mile cotton growing area drained by a single river system (6). A municipal water treatment plant using water from the stream is located in the lower portion of the watershed. An estimated 15,000 acres of cotton are grown annually in the basin, mostly in small plots averaging about 10 acres. Principle insecticides employed are toxaphene (65% of total by weight of active ingredient), DDT (25%) and the gamma isomer of BHC (5%). In 1959 an estimated 79,000 pounds (technical) were applied and in 1960 about 59,000 pounds were used.

1/ Chemical analyses and bioassays in connection with these studies are done by the Department of Agricultural Chemistry, Clemson College, Clemson, South Carolina, that is under contract to the Public Health Service.

Nearly continuous water sampling has been carried on at the water plant by adsorption of insecticides on activated charcoal. Companion samples are taken from both raw and treated water. Gamma BHC was recovered in amounts ranging from 150 to 760 parts per trillion over a period of two months during the late summer of 1959, but not in 1960. A substance, identified as toxaphene on the basis of very strong circumstantial evidence, was recovered with intermittent absences throughout the first 22 months of study. Later samples remain to be analyzed. Recovered concentrations ranged from less than 5 to 150 p.p.t. with largest recoveries occurring during and just after the late summer period of intensive insecticide application.

Available evidence indicates that gamma BHC and toxaphene were present in solution in river water, not adsorbed on sediment; and that they probably reached the river in surface runoff. Furthermore, the contribution was from all parts of the watershed, not just a few strategically located fields. The fact that toxaphene can and does appear in water throughout the year is deemed highly significant and relates to the large quantities of this insecticide applied to the land, its persistence in soil, and its solubility in water. DDT, that is as persistent but not as soluble, was not recovered. The municipal water treatment

process did not remove these insecticides or reduce the quantities present. Current studies are directed toward removal by use of activated carbon in water treatment.

Studies of fish populations, macro-invertebrates and zooplankton were conducted during 1959 and 1960 to determine effects of exposure to these insecticides (7). No adverse results were demonstrated at the concentrations to which these organisms were exposed.

A second study recently completed involves factors related to parathion contamination of a farm pond located in a peach orchard, persistence of that insecticide and the biological effects of that contamination (8,9). It was determined that parathion contamination of water in the pond (0.02 p.p.b.) and pond bottom mud (1.9 p.p.m.) occurred in the spring of 1960 before any use of parathion that year. It was found that the contamination probably occurred in March 1960 during a period of accelerated soil erosion, and that the source of the parathion was the orchard soil where the insecticide had persisted for at least nine months. This changed our opinion of parathion which had been thought to decompose much more rapidly.

Not all problems are related to use of pesticides in agriculture or forestry. The discharge of parathion bearing wastes in May 1961 by an industry manufacturing insecticides resulted in an extensive

local kill of fish and is suspected of affecting fish over 100 miles of stream.

Formulating plants and storage houses also are sources of potential trouble. Most states have a number of these establishments. In the Southeast we had 223 registered formulating or manufacturing establishments in 1957-1958. The explosion and subsequent destruction by fire of one such place in South Carolina in 1961 awakened us to the realization of their potential as sources of water contamination.

Other studies under way in the Southeast involve use of endrin in sugar cane culture, possibilities of ground water contamination in areas of intensive pesticide usage, and studies of selected herbicides persistence in natural waters. As in most such cases, more questions seem to arise than are answered. However, it is believed that a basic need for knowledge is being filled, and that knowledge will have increasing value and application as time passes.

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TOXICITY OF INSECTICIDES TO FISH IN THE NORTHWEST

Max Katz*

The material presented in this paper has appeared in a recent issue of the journal of the American Fisheries Society under the title "Acute Toxicity of Some Organic Insecticides to Three Species of Salmonids and to the Threespine Stickleback." The information presented here is contained in the eight tables which follow this discussion. References are also shown.

These tables contain a record of tests for toxicity of a number of chlorinated insecticides to Northwest cold water fish. The estimated median tolerance limits (TL_m) for chinook and coho salmon and rainbow trout fingerlings to the insecticides tested are listed in Table 1 and are expressed in parts per billion (p.p.b.). Table 2 compares the 96-hour TL_m of chinook and coho salmon and rainbow trout with results reported by Henderson and Pickering for fathead minnows, bluegill, goldfish, and guppy. The TL_m for marine threespine stickleback are shown in Table 3.

Endrin was consistently the most toxic of all of the compounds tested. Co-Ral is the least. Cohos and rainbows

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were generally more tolerant than chinooks except in the case of endrin where the difference was slight. The differences in tolerance of the three species, however, were not large in most cases.

Comparison with the results of Henderson and Pickering shows the salmonids, especially the chinook salmon, to be somewhat more sensitive than the warm-water fish.

The fact that some insecticides are toxic only in relatively high concentrations indicates that useful chemicals can be developed and used that will not be highly toxic to fish.

Table 4 shows endrin to be extremely toxic and that the 96-hour TL_m does not vary greatly for the six species of fish tested.

Table 5 shows that there is little difference in the toxicity of endrin in waters varying in salinity from 5,000 to 27,000. (Sticklebacks were used for the salinity experiment.)

It was found that the volume of water containing the insecticides had an effect on the tolerance of fish. For instance, ten fish in a specific concentration of the chemical contained in one liter died more rapidly than the same number of fish in the same concentration contained in a total volume of 10 liters.

This factor was also demonstrated by the results in Table 6 where the number of fish in similar volumes of water containing the chemical was varied from 5 to 20. This experiment indicated that the fish died in the container containing the largest number. This is important in setting up aquaria experiments, but is not of much practical significance.

Experiments were run using small bluegills and varying the temperature from three to 25 degrees centigrade. Table 7 shows that the toxicity increased with the temperature--being about 30 times that at 25 degrees as it was at 3 degrees centigrade.

Table 8 shows the results of a test for the effects of endrin on embryonic developments. The table indicates that endrin concentrations up to 4.2 p.p.b. did not effect the fertilization and hatching of stickleback eggs. After the eggs hatched the pattern of mortality started to develop. The test indicates that the larvae are more tolerant than the adult fish. This finding substantiates the results of other research and indicates that the egg and larvae stages are not as sensitive to toxicants as are the adults.

TABLE 1

ESTIMATED MEDIAN TOLERANCE LIMITS (TLm) OF SOME INSECTICIDES FOR CHINOOK SALMON, COHO SALMON, AND RAINBOW TROUT

(Expressed In Parts Per Billion Active Ingredient)

Insecticide	Chinook Salmon				Coho Salmon				Rainbow Trout			
	Hours				Hours				Hours			
	24	48	72	96	24	48	72	96	24	48	72	96
Toxaphene	7.9	3.3	2.7	2.5	13.0	10.5	10.0	9.4	11.5	8.4	8.4	8.4
Aldrin	12.4	10.6	8.7	7.5	-	61.0	48.6	45.9	42.4	23.9	20.3	17.7
Dieldrin	7.9	6.7	6.1	6.1	17.5	15.3	14.4	10.8	15.7	13.0	9.9	9.9
DDT	38.0	17.0	14.0	11.5	66.0	46.0	44.0	44.0	42.0	42.0	42.0	42.0
Lindane	56.0	42.0	42.0	40.0	60.0	56.0	56.0	50.0	42.0	41.0	39.0	38.0
Methoxychlor	28.0	27.9	27.9	27.9	66.2	66.2	66.2	66.2	62.6	62.6	62.6	62.6
Heptachlor	32.4	26.6	23.0	17.3	61.9	60.4	60.4	59.0	36.7	33.8	25.9	19.4
Chlordane	59.0	59.0	57.0	57.0	100.0	86.0	82.0	56.0	56.0	44.0	44.0	44.0
Endrin	2.0	1.2	1.2	1.2	1.3	0.8	0.52	0.51	0.79	0.58	0.58	0.58
Guthion	6.8	6.2	4.3	4.3	7.0	5.0	4.8	4.2	4.7	3.8	3.8	3.2
Malathion	24.5	23.9	23.6	23.0	-	-	-	-	-	-	-	-
Co-Ral	-	-	-	-	22,000.0	20,000.0	18,000.0	15.000.0	-	1,800.0	1,500.0	1,500.0
Sevin	-	-	-	-	1,330.0	997.0	997.0	997.0	-	1,600.0	1,350.0	1,350.

TABLE 2

THE 96-HOUR MEDIAN TOLERANCE LIMITS OF SOME INSECTICIDES FOR RAINBOW TROUT, CHINOOK SALMON, COHO SALMON, FATHEAD MINNOW, BLUEGILL, GOLDFISH, AND GUPPY

(Expressed In Parts Per Billion Active Ingredient)

Insecticide	Chinook Salmon	Coho Salmon	Rainbow Trout	Fathead Minnow[1]	Bluegill[1]	Goldfish[1]	Guppy[1]
Toxaphene	2.5	9.4	8.4	7.5	3.5	5.6	20.0
Aldrin	7.5	45.9	17.7	33.0	13.0	28.0	33.0
Dieldrin	6.1	10.8	9.9	16.0	7.9	37.0	22.0
DDT	11.5	44.0	42.0	32.0	16.0	27.0	43.0
Lindane	40.0	50.0	38.0	62.0	77.0	152.0	138.0
Methoxychlor	27.9	66.2	62.6	64.0	62.0	56.0	120.0
Heptachlor	17.3	59.0	19.4	94.0	19.0	230.0	107.0
Chlordane	57.0	56.0	44.0	52.0	22.0	82.0	190.0
Endrin	1.2	0.51	0.58	1.0	0.6	1.9	1.5
Malathion	23.0	-	-	12,500.0[2]	95.0[3]	-	-
Co-Ral	-	15,000.0	1,500.0	18,000.0[3]	180.0[3]	-	-
Sevin	-	997.0	1,350.0	6,700.0[3]	5,300.0[3]	-	-
Guthion	4.3	4.2	3.2	93.0[3]	5.2[3]	-	-

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TABLE 3

ESTIMATED MEDIAN TOLERANCE LIMITS FOR STICKLEBACKS OF SOME INSECTICIDES IN WATERS OF 5 AND 25 PARTS PER THOUSAND SALINITY

(Expressed In Parts Per Billion Active Ingredient)

Insecticide	5 parts per thousand salinity				25 parts per thousand salinity			
	Hours				Hours			
	24	48	72	96	24	48	72	96
Toxaphene	-	12	9.8	8.6	-	-	8.8	7.8
Aldrin	-	48.3	41.5	39.8	-	44.2	27.4	27.4
Dieldrin	20.7	18.9	18.0	15.3	-	-	13.5	13.1
DDT	22.0	21.0	18.5	18.0	18.0	15.0	14.5	11.5
Lindane	50.5	45.0	45.0	44.0	66.0	51.0	50.0	50.0
Methoxychlor	93.6	86.4	86.4	86.4	-	69.1	69.1	69.1
Heptachlor	120.8	111.9	111.9	111.9	111.9	111.9	111.9	111.9
Chlordane	118.0	118.0	90.0	90.0	180.0	170.0	170.0	160.0
Endrin	-	0.45	0.45	0.44	-	-	0.58	0.50
Guthion	15.8	15.8	14.9	12.1	6.9	5.0	4.8	4.8
Malathion	96.9	94.0	94.0	94.0	76.9	76.9	76.9	76.9
Co-Ral	-	2,254.0	1,862.0	1,862.0	-	1,764.0	1,568.0	1,470.0
Sevin	-	16,625.0	6,175.0	3,990.0	-	10,450.0	4,940.0	3,990.0

TABLE 4

TOXICITY OF AN ENDRIN FORMULATION AT 20°C TO RAINBOW TROUT, COHO AND CHINOOK SALMON, GUPPIES, BLUEGILLS, AND GAMBUSIA

TL_m	Rainbow Trout	Coho Salmon	Chinook Salmon	Guppies	Bluegills	Gambusia
24 hr	2.17	12.0	---	---	---	---
48 hr	1.45	0.56	1.50	---	---	1.80
72 hr	1.12	0.30	1.01	1.10	0.95	1.05
96 hr	0.90	0.27	0.92	0.90	0.60	0.75

TABLE 5

TOXICITY OF AN ENDRIN FORMULATION IN WATERS OF VARIOUS SALINITIES AT 20°C TO THE MARINE THREE SPINE STICKLEBACK

	Salinity (part per thousand)			
TL_m	27	22	15	5
48 hr	1.74	2.40	2.25	2.62
72 hr	1.71	1.57	1.71	1.95
96 hr	1.65	1.20	1.57	1.57

TABLE 6

TOXICITY OF AN ENDRIN FORMULATION AT 20°C TO VARIOUS NUMBERS OF BLUEGILLS IN THE SAME VOLUME OF WATER. TOTAL VOLUME 15 LITERS.

TL_m	5 fish	10 fish	20 fish
72 hr	0.95	2.25	2.70
96 hr	0.60	0.86	1.10

TABLE 7

TOXICITY OF AN ENDRIN FORMULATION TO SMALL BLUEGILLS AT TEMPERATURES RANGING FROM APPROXIMATELY 3° TO 25°C

	Temperature (degrees C.)					
TL_m	3 (1.6-4.5)	6 (3.5-8.0)	10	15	20	25
72 hr	---	23.2	---	1.95	2.17	0.36
96 hr	8.25	5.5	2.4	1.65	0.86	0.33

TABLE 8

EFFECTS OF AN ENDRIN FORMULATION ON THE EMBRYONIC DEVELOPMENT, HATCHING, AND SUBSEQUENT SURVIVAL OF MARINE THREESPINE STICKLEBACKS IN WATER OF 10 PARTS PER THOUSAND SALINITY AT 20°C

ppb active ingredient	no. of eggs	% eyed eggs 4 days after fertilization	% eyed fish hatching in 8-9 days	no. of fish hatched	% Survival Days after beginning of hatching					
					3	4	5	6	7	8
Control	38	63	85	20	100	100	85	75	20	5
0.75	32	78	52	13	100	69	61	45	15	0
1.35	21	43	44	4	100	75	75	50	50	0
1.8	27	63	82	13	84	69	61	30	7	7
2.4	22	63	36	5	80	40	20	20	0	0
3.2	24	58	71	10	90	90	10	0	0	0
4.2	26	67	54	13	61	31	0	0	0	0

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TOXICITY OF METALS AND THEIR DETECTION

W. Allan Moore*

The problem of the toxicity of metals to the biota of streams has raised many questions and has been the subject of many papers appearing in the biological field. In relation to these toxicity problems, there is the necessity to differentiate between the physical and chemical characteristics of such waters and the analytical methods necessary to determine the chemical entities which may contribute to or be wholly responsible for the toxicity results obtained.

It is impossible to review here the effects of the various metal ions and their analytical determination to which have been ascribed toxic effects to stream biota. I further believe that it is necessary to point out that no one parameter, whether in the field of biology, bacteriology, chemistry or engineering, can be used to evaluate such toxic effects.

As mentioned previously a great number of metallic ions have been investigated in relation to their toxic effects. In this group may be mentioned copper, chromium, nickel and zinc,

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since these four metals resulting from industrial processes would be expected to appear more frequently and in greater concentrations.

Of the four metals mentioned, copper has received the most attention. In fact, "Standard Methods" established in the 10th edition the maximum concentration of copper at 0.01 mg. per liter in their "Standard" dilution water used for the BOD test. Perhaps this concentration may be too high or low, but the experimental results so far published have not presented any conclusive evidence that this is the minimum or maximum copper concentration which will affect the BOD test. There can be a vast difference between the amount of copper ion added and its ionic concentration in solution to which may be ascribed toxic effects. The physical and chemical characteristics of the particular substrate used, will control to a large extent the concentration of the metal ion present.

It would seem natural, therefore, that a difference in toxicity can be obtained between waters having a high pH value (soft waters) and those having a lower pH value (hard waters). The concentration of organic components present may also effect the toxic limits of metallic ions. Of those industrial processes involving the plating of copper, the plating solution

used is usually the copper cyanide complex in an alkaline solution. However, in pilot plant studies using the activated sludge process, the removal efficiency of BOD showed little difference when the copper was fed as the sulfate or as the complexed cyanide in equivalent copper concentrations. This immediately brings up the question of the analytical determination of the various physical states in which copper may be present. In a given substrate copper may be present as the insoluble, colloidal, or as the soluble metal ion. The total copper can be determined by first destroying the organic material, adjustment of pH and development of color by using either the cuprethol or the cuproine methods. In using the former reagent, it is necessary to adjust the pH to 5.2 ± 0.1. This is best accomplished by using a phthalate buffer instead of the acetate buffer recommended in "Standard Methods."

In using the "cuproine" method less rigid control of the pH is necessary and this reagent is specific for copper and can be used in the presence of nickel, and chromium, whereas, with the cuprethol method the presence of either of these two elements may cause interference. For high concentration of copper greater than 10 mg. per liter, a volumetric method is used. In this procedure the iron is complexed as the fluoride, potassium iodide added to the acidified solution and the liberated iodine

titrated with standard thiosulfate. When the soluble and colloidal copper concentrations are to be determined, the sample is first filtered through a millepore filter. The soluble copper may be determined by either the cuprethol or cuproine methods directly. The total copper in the filtrate (colloidal plus soluble) is determined by wet combustion and the difference between this and the soluble copper is equal to the colloidal copper present. It is the concentration of soluble copper to which may be ascribed the toxic properties of this metal.

In plating solutions in which chromium is used, the metal in these solutions is in the hexavalent state. The waste discharged from such operations will not only contain the excess hexavalent chromium, but also the trivalent form. When such acid wastes are discharged to a sanitary sewer, the excess acidity is neutralized and the trivalent chromium is precipitated as a hydrated oxide ($Cr_2O_3.^X H_2O$) and rendered innocuous. It is, therefore, with the hexavalent form that we are particularly concerned. A portion of this hexavalent chromium will be reduced to the trivalent state by the organic material present in the sewered waste. It has been found that concentrations of hexavalent chromium as high as 50 mg. per liter will not appreciably affect the activated sludge process when fed on a con-

tinuous basis. Even when a slug dose of 500 mg. per liter of hexavalent chromium was fed, the pilot plant recovered after four days.

The analytical determination of chromium in various substrates is not particularly difficult. However, if chlorides are present (concentrations greater than 100 mg. per liter), the chromium must first be reduced with sulfite during the oxidation of the organic matter present. The reduced chromium is oxidized to the hexavalent state with $KMnO_4$ and reacted with diphenylcarbazide. Soluble chromium (hexavalent) may be determined by filtering the sample through a millepore filter and determining the chromium in the filtrate directly.

It has been found that the toxicity of zinc (a commonly occurring metal) is about the same regardless of whether the metal is fed as the sulfate or as the complexed cyanide. Its analytical determination, however, is not as simple as that of copper and chromium. Many analytical methods have been proposed, but the comparatively new method in which "zincon" is used has been found to be satisfactory in many cases. However, in certain substrates this method leaves much to be desired and the rather tedious polarographic method has been utilized.

Nickel has not proven to be as toxic as some investigators have claimed. As with many of the other heavy metals, the physical and chemical characteristics of the substrate will determine the concentration of nickel ion in solution. For example, in the primary settler of the activated sludge system anaerobic conditions are encountered with the subsequent formation of sulfides. The presence of such sulfides will remove a portion of the nickel in solution and render it non-toxic.

The colorimetric determination of nickel in the past was carried out by using dimethylglyoxime. Due to the fact that wide variations can be obtained by this method, a more sensitive and reliable reagent was sought. The compound, α - furil dioxime was found to meet the necessary criteria and today is the reagent of choice. High concentrations of nickel are best determined volumetrically with "versenate" using verichrome Block-T as an internal indicator. In this method the nickel must be precipitated twice as the glyoxime, dissolved and titrated with the above reagent.

DISCUSSION

Q. In the determination of zinc was the test made directly on the water or was it necessary to concentrate before testing?

A. It was necessary to concentrate.

Q. At what concentration can a direct polarographic reading be made for zinc?

A. I think you can get down to less than a part per million if you use concentration, but I do not know how low a concentration can be determined by a direct reading.

Q. You mentioned that it was possible to distinguish between dissolved and colloidal copper. I presume by "dissolved" you mean "ionic" copper. How do you separate the dissolved from the colloidal?

A. First, we test for total copper (after destroying the organic material). Then we filter another aliquot portion of the sample through a millepore filter which will take out all of this suspended copper. The colloidal and soluble copper pass through the filter. The colloidal copper will not react with cuprethol or cuproine. Therefore, the amount of soluble copper can be determined in the filtrate from the millepore filter. Total copper is determined using the same filtrate. You can get the colloidal copper by difference.

Q. Was this colloidal copper in the form of a carbonate?

A. No, it was in the form of cupreous oxide.

Q. What about precipitating copper sulfide from a cyanide complex?

A. Most textbooks will say that the sulfide cannot be precipitated from the copper complex. Unfortunately, this is not true. At a pH of 10 no sulfide is obtained from a complex, but, if the pH is lowered to 9, a small amount of sulfide is precipitated, and at a pH of 7.5 the copper will precipitate in the form of a sulfide. Therefore, the cyanide complex is broken up in a pH above 7.0.

PULP MILL WASTES VERSUS OYSTERS

R. E. Dimick*

There is need for standard methods by which to evaluate the effects of pulp mill wastes on oysters, particularly in the range of concentrations which may be slowly lethal or sub-lethal. Variations in the results of bioassay reported by different sources may be due to differences in techniques, characteristics of waste samples employed, and to other factors.

Bioassay procedures with oysters should encompass the several life-history stages or phases such as spawning, fertilization, embryonic development, pelagic larvae, growth and general condition of the adults. It is generally recognized that oyster bioassay methods should provide for the evaluation of injurious effects of waste concentrations far below those causing rapid injuries or mortalities in other test animals. Recent investigations in several laboratories indicate that the embryonic stages of some bivalves are particularly susceptible to a variety of toxic substances. These animals, therefore, present a promising possibility as test animals for short-term (2 to 48 hours) bioassay assessments of toxic or non-toxic properties of wood processing wastes.

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Some of the results obtained at the Yaquina Bay Laboratory indicate that variations in the characteristics of the pulp mill waste samples employed may have resulted in significant differences in the apparent damages to oyster larvae. This suggests that consideration be given to the use of waste samples which are similar in characteristics to the wastes encountered in receiving waters, especially those occurring on or near oyster grounds.

Rates of flow may cause variations in bioassay. In case of long-term (8 to 9 months) continuous flow exposures to spent sulfite liquor concentrations in which the effect on adult oyster mortalities was not clearly demonstrated, it was found that two liters per minute solution flow was not adequate to maintain 100 oysters per test tray in approximately equal condition. Condition factors decreased from front to rear in the test trays. Solution flows in current bioassays have been increased to four liters per minute per 100 test oysters (Natives and Kumamotos) and five liters per minute for 25 Pacifics. (Food supply is undoubtedly involved in the above variation.)

Another problem in long-term oyster bioassays which has been encountered is the high mortalities occurring with Native

oysters in the control groups. These mortalities range in different years from 10 percent in 6 months to 24 percent in one year. On the other hand, mortalities in Kumamotos and Pacifics have been extremely low (0.0 to 7.0 percent), usually about two percent in 8 months. A reliable method should be devised to assess significant differences in Native-oyster mortalities occurring in SSL concentrations ranging from 10 to 200 p.p.m. during testing periods of 8 months to one year. There appears to be no problem with this species in high SSL concentrations (500 to 2000 p.p.m.) for differences in mortality rates in relation to increases in SSL concentrations become evident within 70 to 80 days.

In the case of Kraft mill effluents, the main problem is conducting long-term oyster bioassays appears to be that mill waste samples even from the same mill vary in toxicity from time to time, and in the same sample with time and differences in storage conditions. Perhaps there is need for a prepared or synthetic standard Kraft mill effluent for test and comparative purposes.

DISCUSSION

Q. Did you test any of the components in the kraft liquor?

A. No, however, the mill did give us a list of some of their

chemicals, along with other information regarding the waste that is discharged.

GRANTS PROGRAM OF THE PUBLIC HEALTH SERVICE

Ralph H. Holtje

(Note: Mr. Holtje of the Research and Training Grants Branch of the Public Health Service, Washington, D. C., described briefly the new arrangements for administering research and training grants through the Division of Water Supply and Pollution Control.)

Congress within the last few months has made moneys available for fellowships and training grants to individuals, training grants to institutions, and for demonstration grants. A description of each of these types of grants can be obtained by a request to Research and Training Grants Branch, Division of Water Supply and Pollution Control, Public Health Service, Washington 25, D. C.

The details of the procedures to be used in administering this grant program are presently being developed in the Washington office. Appropriations have been made for research grants for 1962 in the amount of $2.67 million. This is for research in universities and other organizations outside of the Federal Government. Appropriations for fellowship grants amount to $100,000, which will take care of about 20 fellowships during this year; for training grants $900,000, which are used for adding to the faculty and facilities and strengthening the

curricula of colleges and universities; and for demonstration grants the amount is $400,000. The latter type of grant is to bridge the gap between the obtaining of new knowledge and putting it into use.

FISH TOXICITY STUDIES ON ATOMIC PROCESS WASTES*

P. A. Olson**

At the Hanford Works large volumes of Columbia River water are passed through the nuclear reactors as a coolant and are ultimately returned to the river. This effluent constitutes a potential hazard to aquatic life since it contains certain toxic chemicals, is heated and is mildly radioactive. Investigations of the effect of this effluent on aquatic life is one of the research activities of the Biology Laboratory.

This presentation reviews the results of some of the past laboratory experiments. Young salmon were the primary test animals for these studies since they represent a species of economic value and also appear to be among the forms most sensitive to the effluent water. Because of easier availability most of the stocks tested were of Puget Sound origin and these are somewhat more sensitive to the effluent than those of Columbia River origin (1). The studies were designed to determine the effluent concentrations at which adverse effects were

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apparent and to evaluate the separate toxic components involved. The studies were run on a chronic basis characteristically covering the early life period of the salmon in fresh water from the time of fertilization of the eggs until the seaward migration of the fingerlings. Experimental levels or conditions in these laboratory tests causing adversity to the young fish exceed existing river conditions by a substantial margin.

Effluent Monitoring

Table 1 summarizes the results of a test exposing young chinook salmon to effluent water from November when the eggs were fertilized until June when the young fish were ready to migrate. Concentrations of 5 per cent and greater resulted in increased toxicity.

Table 1

Mortalities and growth of young chinook salmon exposed to different concentrations of reactor effluent

Condition	Per Cent Mortality				Average Weight (g)
	Egg	Fry	Fingerling	Total	
Control	12.2	4.0	2.5	18.7	1.8
1% reactor effluent	11.9	2.9	4.0	18.8	2.3
2% reactor effluent	12.0	2.4	2.6	17.0	2.8
3% reactor effluent	13.0	2.0	4.6	19.6	3.2
5% reactor effluent	13.1	5.0	8.1	26.2	4.2
10% reactor effluent	17.0	46.6	29.5	93.1	5.3

Far more young salmon encounter the Hanford Section of the Columbia as brief transients (upriver fingerlings on their ocean migration) than as progeny arising from spawning in this immediate section. Young fish which are exposed to the effluent for the first time as fingerlings are far more effluent resistant than when exposed at younger life stages. This has been demonstrated by experiments in which both chinook and blueback fingerlings have been exposed to concentrations as great as 10 per cent effluent for several months without demonstrating any adverse effect (1,2,3).

Until recently the concentration of effluent in the river remained relatively stable throughout the day but, of course, fluctuated seasonally with changes in river flow. However, since the completion of a power dam just upriver from the Hanford Project, the river flow is no longer stable throughout the day; it may be 2.5 times greater during the afternoon and evening when the power load is highest than during the early morning when the load is minimal (4). Studies carried out to determine whether or not these diurnal fluctuations would increase the toxicity of the effluent to Columbia River fish involved exposing young chinook salmon to three types of flowing water conditions between mid-November and early June (5).

No effluent was added to the water supplied to the control group; in the second case enough effluent was added to make a 4 per cent strength solution since this was expected to produce some toxic effect; and in the third case, which simulated fluctuating river flow, effluent was added according to the following pattern:

Time of Day	Per Cent Effluent
3 a.m. to 9 a.m.	6.2
9 a.m. to 11 a.m.	3.8
11 a.m. to 11 p.m.	3.0
11 p.m. to 3 a.m.	3.8
Avg. for 24-hour period	4.0

The results are summarized in Table 2.

Table 2

Comparative toxicity of reactor effluent under fluctuating conditions

Condition	Per Cent Mortality				Average Weight (g)
	Egg	Fry	Fingerling	Total	
River water	2.4	0.9	3.1	6.4	1.0
4% effluent--steady	2.7	1.4	10.4	14.5	1.8
3 to 6.2% effluent--fluctuated	2.3	2.1	8.3	12.7	2.0

The increased toxicity of the 4 per cent effluent over that of the river water was evident. The fluctuating concentration of effluent did not increase the toxicity over that of the 4 per cent effluent administered at a steady rate.

Toxicity of Hexavalent Chromium

The toxic effect to fish from high concentrations of effluent has been attributed in part to the presence of sodium dichromate. Chronic exposure tests of young salmon and trout to hexavalent chromium have demonstrated slight toxic effects at concentrations approaching 0.02 ppm and marked toxicity at 0.08 ppm (6).

In addition to such chronic tests as above, a test was run to determine if intermittent exposures involving the same total amount of dichromate over a period of time would affect fish more or less than the chronic exposure (7). Since chronic exposures to fish of 0.08 ppm Cr(VI) are quite toxic (6) similar exposure levels were used to study the relative effect of intermittent exposures compared to chronic. Newly spawned chinook salmon eggs were placed in each of three troughs under the conditions shown below. The experimental design was such that each lot was exposed to the same total amount of Cr(VI) over a two weeks' period. The experiment was continued for seven months until the young fish were of sufficient size and age for migration to the ocean.

ppm Cr(VI) Added

0.07 (continuous)

0.14 (alternate weeks)

0.49 (one 24-hour period per week)

None (control)

The following summarizes the results of the test:

Table 3

Mortalities and growth of young chinook salmon exposed to chronic or intermittent levels of hexavalent chromium

ppm Cr(VI) Added	Per Cent Mortality				Average Weight (g)
	Egg	Fry	Fingerling	Total	
0.07 (continuous)	6.5	4.5	43.3	54.3	0.76
0.14 (alternate weeks)	6.4	7.9	35.3	49.6	0.89
0.49 (one 24-hour period per week)	7.3	1.9	27.4	36.6	0.94
None (control)	6.9	2.5	3.2	12.6	1.96

No significant difference in mortality occurred during the egg stage. After hatching, approximately six weeks passed before the death rate began to increase. At the end of the fry stage, poorest survival was in the lot which received 0.14 ppm (alternate weeks) and best survival was in the lot which received 0.49 ppm (one 24-hour period per week). At the conclusion of the test, significantly fewer fish survived in the 0.07 ppm (continuous), and best survival was in 0.49 ppm (one 24-hour period per week).

The growth results are in agreement with the mortality data. At the end of the experiment the smallest fish were in the chronically exposed lot and the largest were in the lot exposed for 24 hours each week.

For this experimental design the chronic release of dichromate was slightly more toxic to aquatic life than if the same quantity were released intermittently.

Temperature Tolerance Study

A study was made on the temperature tolerance of eggs and young of chinook salmon which spawn in the main stem of the south central portion of the Columbia River (Priest Rapids locality) (8). This section supports a fall run which spawn between the middle of October and the middle of November. In general, the river temperatures range from the mid to high fifties during major spawning.

A pair of ripe chinook salmon (local stock) was captured on the spawning grounds. Eggs were taken and the progeny subjected to a temperature test from time of capture of October 26 until the following May. The control temperature followed a seasonal trend typical for the locality. It started at 57F, reached a minimum of 36F and increased to 47F at the end of the test. Other experimental lots averaged 2F, 4F and 8F warmer

than the control throughout the greater part of the test. Table 4 presents the summary of results.

Table 4

Mortalities and growth of Columbia River chinook exposed to different temperature conditions

Initial Temperature	Per Cent Mortality				Average Weight (g)
	Egg	Fry	Fingerling	Total	
57F	7.9	1.8	6.3	16.0	1.0
59F	6.2	1.0	2.8	10.0	1.3
61F	8.7	1.6	0.0	10.3	2.2
65F	10.8	40.9	27.2	78.9	4.1

Consistent mortality above that of the control occurred only in the warmest lot. The results of this single experiment indicated that the eggs could begin incubation at temperatures as high as 61F without significant loss.

Radioactivity

The quantities of the various radionuclides which accumulate in the different species of fish is dependent primarily on the feeding habits of the fish and the metabolism of particular nuclides and not so much on their abundance in the water. The adult salmon which do not feed after leaving the ocean on their spawning migration remain virtually uncontaminated. Suckers, which

feed directly upon such material as algae, usually contain higher concentrations of nuclides than species such as bass which obtain the isotopes third or fourth hand via other food organisms.

Although a variety of radionuclides have been found in Columbia River fish, over 90 per cent of the radioactive material is P^{32} (9). There is a seventy-five fold difference in concentration of radioactive materials in fish between winter and late summer (10). The lower levels during the cold season of the year are attributed to reduced consumption of radioactive food organisms and slower metabolic rates.

Although a number of tests have been run to follow the uptake and deposition of several isotopes in both marine and fresh-water fish, there is little direct information on the concentrations of deposited isotopes which produce significant radiation damage to fish. Some local laboratory data are available on the concentration of radiophosphorus in fish which is tolerable. Damage was not detected in cichlid fish with an average concentration of 0.09 μc P^{32}/g fish after nine months' exposure to the isotope. Another experiment showed slight radiation damage in trout tested for six months and having concentrations of about 0.42 μc P^{32}/g fish (11). From these data one might infer that the threshold concentration of P^{32} causing some damage lies somewhere between

these two values. The lower value exceeds that of sampled Columbia River fish by an order of 100.

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AN EXAMPLE OF THE TOXIC EFFECTS OF ORGANIC DEPOSITS

Charles D. Ziebell*

Introduction

Today we have heard some fine presentations pretaining to pesticides, insecticides, metals, and pulp mill wastes, and the part they play in creating toxic conditions to various fish and shellfish. My specific topic, AN EXAMPLE OF THE TOXIC EFFECTS OF ORGANIC DEPOSITS, does not lend itself to specific evaulation as some of the previously mentioned toxicity studies have done. However, a relationship does exist between the aforementioned toxic substances and organic deposits even though it is not always evident on the surface.

Controlled laboratory experiments are excellent guide lines from which to derive certain conclusions. In an ever-changing situation in the field, as in an estuary, one must be cautious in order not to make precarious or erroneous conclusions since results of experiments are not always as defined as in controlled situations.

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With this in mind, let us begin to consider briefly what organic deposits we might normally expect in a marine environment. Bader (1954), in his study of marine sediments, found that a series of bottom samples revealed essentially dark mud with variable amounts of organic matter ranging from about 1 to 8 per cent. He also found up to a certain point, organic deposits were beneficial to pelecypods. Many pelecypods actually utilize organic material from detritus for food. He also states that above 3 per cent organic content the products of decomposition and/or the decline in available oxygen become limiting variables and thus population densities decrease.

So, speaking in terms of environmental suitability--in this case for pelecypods--a certain amount of organic material deposited on the bottom can be considered normal and beneficial. However, excessive organic deposits can create situations that have deleterious rather than beneficial effects.

Microbiological decomposition of organic matter in sediment occurs in marine environments. Waksman and Starkey (1931) have shown that natural decomposition of organic matter can produce aldehydes, hydrogen sulfide, and many other toxic products.

Waldichuk (1959) stated that the build-up of materials which settle out from pulp mill wastes contributes to a change

in the bottom ecology. The organic wastes blanket the bottom of marine waters and render them unsuitable for the development and growth of desirable marine benthic organisms. Populations of bottom dwelling animals gradually shift from the sensitive species to more tolerant forms, and as conditions deteriorate further even these forms may not be able to survive. In general, this gives us some background information with which we can basically differentiate between normal and abnormal organic deposits and the conditions pertaining thereto.

Origin of Abnormal Organic Deposits

There are several ways in which abnormal organic deposits get into waterways to produce sludge beds. One common source is that of municipal sewage. Wood fibers lost from pulp and paper making processes cause sludge beds in many areas as these fibers sink to the bottom. Sawdust from log pond saws, without proper collection units, are another means by which sludge beds can be formed. Cannery and frozen food waste solids, if not properly collected, can cause abnormal organic deposits in a waterway. These are just a few of the more common sources of organic waste materials which can create sludge bed problems in waterways in our constantly growing and developing civilization.

Sludge Bed Formation, Decomposition, and Effect Upon Salmonid Fishes

With the introduction of extraneous organic deposits, eventually sludge beds are formed and changes in the environment are inevitable. Aerobic decomposition of the organic material takes place followed by anaerobic decomposition when the dissolved oxygen supply is exhausted. When anaerobic decomposition occurs, organic material is reduced and eventually sulfides, mercaptans and methane gas are the products.

Sulfides have been demonstrated to be toxic to fish at very low concentrations, particularly fish in the family salmonidae. In addition to toxicity, other factors must be considered when decomposing organic deposits are involved. In a fairly recent study which the Pollution Control Commission made, a problem was encountered that was not as clearly defined as an ordinary toxicity problem. A report was received that downstream migrant steelhead were seen in distress in a small estuary near a particular industry. Initial investigation substantiated this report and our first observations during low tide led us to suspect that excessive wood fiber deposition would be a factor in this particular problem. Subsequent water quality analysis confirmed, in part, our suspicions. Sulfides above 1 ppm were detected in several areas of the estuary.

The next thought was to test the fish in live boxes in areas above and below the industry to determine whether or not an acute toxicity problem actually did exist. These live box tests were conducted at different stages of the tide. A site to place a live box was chosen in a narrow passageway below the industry. All fish migrating seaward would have to go through this area in order to get to Puget Sound. The live box was anchored to the bottom of the channel and migrant size steelhead were placed in it. Three hours and 20 minutes later all of the fish were dead. Tests above the industry revealed no mortalities.

Taking into account the rapid mortalities demonstrated in the live box experiment below the industry, there was some doubt that sulfide was the only toxic substance involved.

Consequently, polyethylene test aquaria were brought to the estuary and water containing industrial effluent that had seeped through exposed sludge beds was collected from the main channel. This time the location was approximately 200 yards closer to the outfall of the industry concerned. Test fish were placed in the test aquaria shortly after the sample had been taken. Within three minutes the fish in this aquarium passed through a state of equilibrium loss and lay immobile on the bottom. In 15 minutes all of the test fish were dead. Temperatures were checked and

dissolved oxygen determinations were made concurrently. Both were within tolerable ranges.

This rapid mortality came as somewhat of a surprise. The difference in the length of time until total mortality of fish in the live box test and those in the aquarium bio-assay was much greater than had been anticipated.

Our next move was to make in-plant investigations and then conduct controlled bio-assays with effluent from the industry. While investigating in the plant it was learned that bactericides containing chlorinated phenols and mercury were being used, and that these materials made up a portion of the effluent. Bio-assays using the industry waste water demonstrated an acute toxicity problem. But again, these results were not comparable to any other tests, since these test fish died in 42 minutes. After this series of experiments we felt we had determined the origin of the toxic materials, and the stage of the tide when we could expect rapid mortalities. We also decided that the possible reason for the difference in time until death between fish in the aquaria and live boxes was neutralization of some of the reducing compounds, a function of dilution, mixing, and aeration, and dilution of the mill waste water. There was no doubt at this point that the source of a toxic substance had been

located but one question was not answered. It was still not definitely known why the time until death between the bio-assays with the industry waste water and the tests with estuary water below the outfall were so incomparable.

After a review of the test data and after further observations of the physical situation were made, it was assumed that a change was experienced as the industrial effluent flowed over the decomposing sludge beds. As the already acutely toxic waste water channeled its way over and through the decomposing wood fibers during a low tide, a chemical change occurred to produce a more highly toxic water. One possibility could be the presence of phosphine or its compounds had leached from recently deposited fibers.

Circumstances such as these are quite often overlooked. There are times when both effluent and receiving water analysis may not indicate trouble, but at the bottom of a river or estuary something else may be taking place. Unseen synergistic effects may put a different light on many problems.

Conclusions

There could be many situations such as was experienced here that are not always evident or even detectable through chemical analysis or other means. There could be many of these situa-

tions existing right now where on the surface everything is apparently under control. Effluent analysis may reveal that conditions should propose no problem. But are we on firm ground when we make conclusions based on these analyses without actually knowing what happens under the surface? I sometimes wonder. Is it possible that there are more instances where synergism or chemical changes have created problems of acute or chronic toxicity? Have sludge beds and decomposition of organic material with their resulting production of sulfides and other products coupled with a synergistic affect, created problems unseen on the surface? Have our salmonoid fishes, which by the way, have a pneumatic duct to the air bladder and sink when they're dead, been aff ted without our seeing or knowing it? Gentlemen, these are some of the things to think about. Too often we have a tendency to overlook potential problems. Honestly sometimes, but nevertheless unwisely. Perhaps we should try to project our thinking toward the future. I'm sure we will uncover many things that have been overlooked in the past.

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TOXICITY VERSUS AGRICULTURAL PLANTS AND SOILS

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Introduction

Terrestrial plants use the soil as their root hold and as the main source of all nutrients except carbon which comes from the atmosphere. The soil must provide water for growth and as a medium from and through which other nutrients are absorbed. Plant growth is possible only when the active portion of the plant's root system is bathed in water. It can easily be demonstrated that terrestrial plants operate entirely in an aquatic environment. This aquatic environment is represented by soil solution--the solution which occupies the voids in the soil. The soil scientist concerned with plant nutrition studies the soil solution in much the same way as the limnologist or oceanographer studies the waters of streams, lakes, and the ocean. The chemistry of the soil solution is somewhat more complex than the chemistry of stream, lake, or sea waters because of the powerful influence of the soil solids, but the basic concepts do not differ greatly. It is worth noting that

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range of ionic concentrations in the soil solution overlaps the range of ionic concentrations in waters of interest to both the limnologist and the oceanographer.

The Soil Solution as the Aquatic Environment of Plants

The soil is a three-phase system--solid, liquid and gas. The liquid and gaseous phases occur in the pores of the solid matrix, and are reciprocally related. As the moisture content (soil solution) is depleted by plant use or evaporation, the air content of the soil increases. The soil solution exists as films around the soil particles, microorganisms and plant roots in the soil. The higher the moisture content, the thicker are these films. The soil solution always contains dissolved solids. The quantity and composition of the salts in the soil solution depends upon a host of factors: the input of salts by rain or irrigation water, the weathering rate of soil minerals, the extent of removal of nutrients by cropping, and the leaching and drainage conditions in the soil are prominent examples. The concentration of salts thus depends upon the net input-outgo balance and upon the water content of the soil.

Plant roots ramify through the soil and are bathed by the soil solution. Plants obtain water, oxygen and all of the 12 essential mineral elements from or through the soil solution.

As the moisture content of the soil decreases, plant growth slows and finally ceases because of inability to meet evapotranspiration requirements for water and because of restricted nutrient uptake. Microbiological processes are similarly affected. The soil solution thus plays a central role (Fig. 1), and its composition reflects the current status of a wide variety of forces.

The composition of the soil solution can be used to indicate proportions among exchangeable cations on the clay and humus colloids (22), and to predict existing nutrient conditions for plants with respect to nitrogen, sulfur and phosphorus (7, 8, 15).

Soil chemists have used many techniques to study the soil solution and its role in plant nutrition. There is no satisfactory means for obtaining reasonable volumes of the soil solution as it exists under field conditions; the amounts extractable, even under high pressures, are small (22). As a compromise, extracts are made of the soil after water has been added to the saturation point, that is when the air has been displaced and the pore space is full of water. When moistened to the saturation percentage, all soils are about equally wet in the sense that the moisture content will be about twice the moisture content of the soil at field capacity--the condition which obtains after 1-3

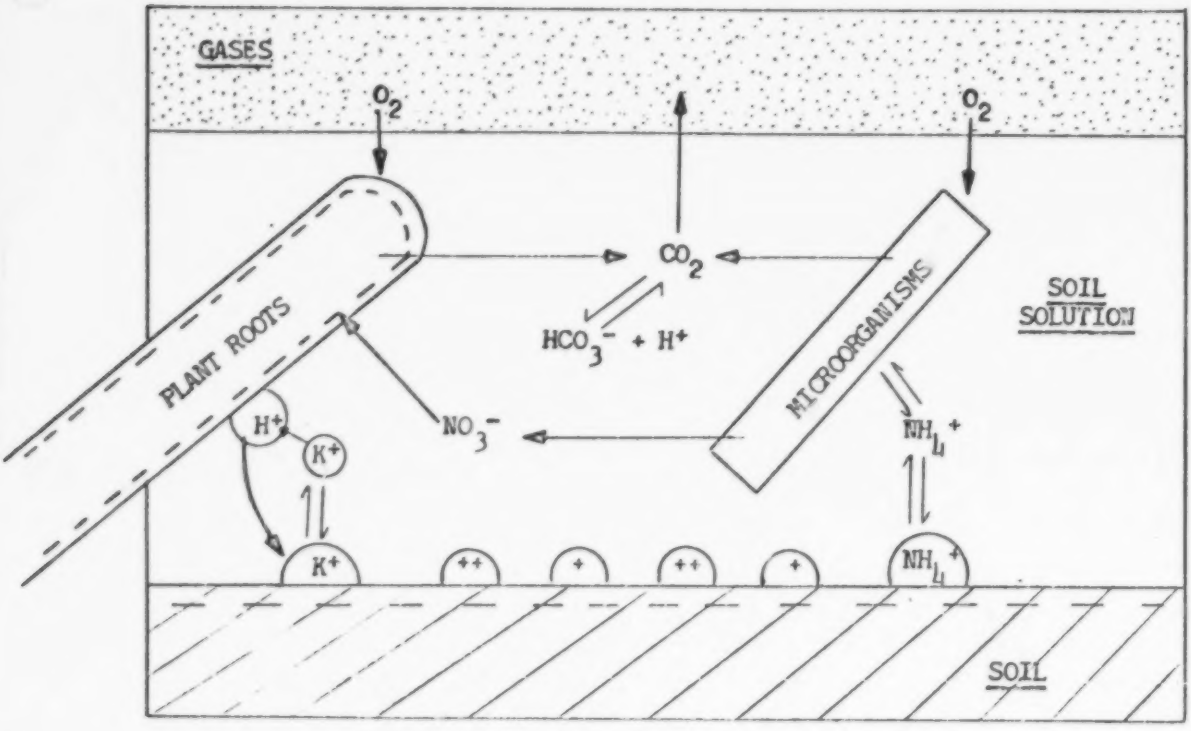

Fig. 1 -- The Central Role of the Soil Solution

Exchangeable cations sorbed on the negatively-charged soil and plant roots equilibrate with the same cations in solution. Plant roots and microorganism's select certain nutrients and reject others. The distribution of ions within the soil solution is heterogeneous; cations are positively adsorbed on negatively-charged surfaces whereas anions are negatively adsorbed.

days of free drainage following a rain or an irrigation. The concentration and composition of the saturation extract serve as indexes of conditions in field-moist soils which are significant to growing plants and provides a basis for comparing soils of very different moisture-holding capacities (22).

The concentration of ions in the saturation extract, as in irrigation waters, is simply indicated by conductivity (22). The unit of conductance most widely used in soils work is the millimho/cm.; in this paper, the micromho/cm. will be used because of its wide employment in water quality studies.

For most soils, the concentration of the saturation extract is usually less than 1000 micromhos/cm.; above this point concentration and composition begin to adversely affect plant growth.

Concentrated soil solutions occur generally under two conditions: (1) where drainage is restricted and (2) where saline waters are used for irrigation. For the diagnosis of detrimental effects of soil salinity or the potential detrimental effects of irrigation waters on soil properties and crop growth, three attributes of the saturation extract of the soil or of an irrigation water are generally considered to be important:

1. Total Concentration (Salinity Hazard) -- expressed in

terms of conductivity or meq./l.

2. Proportion of Sodium to Other Cations (Sodium Hazard) -- expressed in a number of ways: soluble sodium percentage (SSP), sodium absorption ration (SAR), and residual sodium carbonate (RSC) are examples.

3. Specific Toxic Effects -- commonly expressed as p.p.m. or meq./l.

Toxicities Caused by Salinity and Excessive Sodium

The Salinity Hazard is a function of salt concentration and operates in one of two ways: (1) to prevent germination and emergence of seedlings (1,2,3,5,12) and (2) to increasing total moisture stress and hence to reducing the amount of moisture available for uptake by plants (5,12,13,20).

Where salinity is high in the seed bed, erratic germination is common; if salinity is extreme, the land may be barren. Most plants are more sensitive to salinity during germination than at later growth stages. The usual result, therefore, of soil salinity is a partial stand of the crop plant and a much reduced yield. Through proper placement of irrigation furrows with respect to seeds rows (4, 13) or through bed design (2,3,4), it is possible to irrigate in such a way as to reduce the salinity hazard during germination and emergence and thereby ensure

a good stand of plants and a reasonable crop yield.

The diagnosis of salinity hazard is based on the measured conductivity of the saturation extract of the soil or of irrigation water. From numerous observations of crop behavior a scale of salinity can be established. Table 1 is the scale most widely used in the United States. Figure 2 illustrates one scheme for interpreting the conductivity of irrigation waters. It should be emphasized that Table 1 and Figure 2 represent interpretive guides (19,22,23) which, although widely used, are subject to modification. Other diagnostic schemes and guides have been proposed and used. The experienced diagnostician uses all the information at hand and considers other modifying factors such as soil characteristics (texture, structure, etc.), soil and irrigation management, and drainage when assessing hazards from salinity or any other source.

The Sodium Hazard operates in a variety of ways. Cations in solution equilibrate with exchangeable cations on the soil colloids (clays and humus). In normal, productive soils the cation exchange system is dominated by Ca^{++} and Mg^{++} and the soil physical condition is such that water enters and moves readily in the root zone and gaseous interchange is rapid; plants are able to develop profuse root systems capable of active respiration

TABLE 1

Scale of Conductivity of the Saturation Extract of Soils in Relation Crop Response (22)

Conductivity of Sat. Ext. /mhos/cm.	Crop Response
0 - 2,000	Salinity effects mostly negligible
2,000 - 4,000	Yields of very sensitive crops may be restricted
4,000 - 8,000	Yields of many crops restricted
8,000 - 16,000	Only tolerant crops yield satisfactorily
16,000 - ?	Only a few very tolerant crops yield satisfactorily

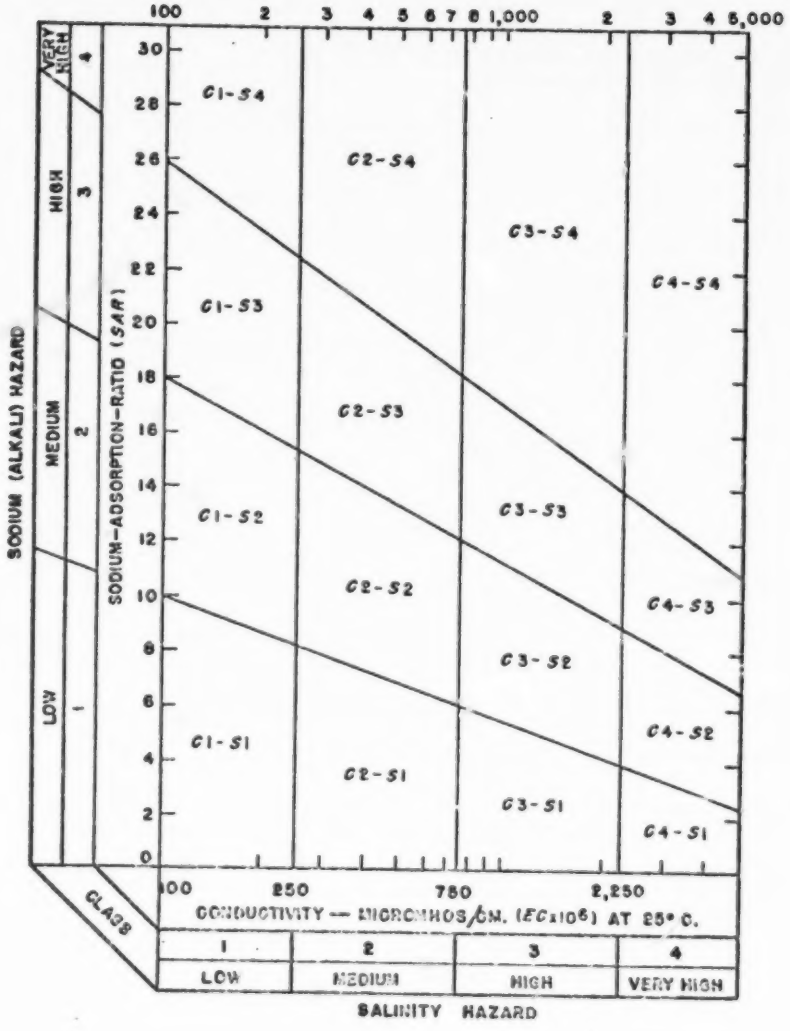

Fig. 2 -- Classification of Irrigation Waters Based on Conductivity and Sodium Adsorption Ratio (22,23)

and nutrient absorption. Upon the addition of moderate amounts of salt (salinization), desirable physical conditions are maintained as long as Ca^{++} salts dominate. When the proportion of Na^{+} salts in the soil solution increases markedly, Ca^{++} and Mg^{++} are displaced from the exchange complex by Na^{+}, soil physical condition deteriorates, and a structure adverse to plant growth results. Soils with much exchangeable Na^{+} (sodic or alkali soils) may become dispersed and puddled and, as a result, poorly aerated. Under these conditions both water absorption and nutrient uptake by the plant are restricted. If the exchange complex becomes more than 40-50% saturated with Na^{+}, nutritional disturbances are evident. Under most circumstances, high alkalinities (pH 8.5-10.0) are associated with high levels of exchangeable Na^{+}. The end result is a severe nutrient imbalance; extremely low levels of available Ca^{++} may occur in sodic soils which contain as much as 20% $CaCO_3$. Other deficiencies are also notes: Fe deficiencies are common and P and Zn deficiencies have been observed.

Crop failures on sodic soils are chiefly due to germination and emergence failures--as is the case for saline soils. Most sodic soils are also saline (saline-sodic). However, germination and emergence failures on saline-sodic are more often due

to poor physical condition than to salinity. Because of the dispersed nature of the sodic soil, water does not infiltrate readily with the result that the seeds may not get sufficient water to permit germination. A common hazard with sodic soils is the formation of harsh surface crusts with sufficient mechanical strength to prevent emergence of seedlings; these crusts form as the soil surface dries following a rain or an irrigation.

The diagnosis of Sodium Hazard via analysis of the soil solution or the irrigation water is effected in several ways. On soil solutions, two ion ratios are widely used to indicate the hazard: the soluble sodium percentage (SSP) and the sodium absorption ration (SAR). The SSP is simply the percentage ratio of Na to other cations in solution.

$$SSP = \frac{Na \times 100}{Na + K + Ca + Mg} \text{ (meq./l.)}$$

The SAR is a more useful value because it accounts for the differences in behavior of monovalent and divalent cations in the soil-water system. The U. S. Salinity Laboratory (22) has proposed this value:

$$SAR = \frac{Na^{+}}{Ca^{++} + Mg^{++}/2} \text{ (meq./l.)}$$

because it can be used with considerable reliability to predict the degree to which Na^{+} saturates the exchange complex of the

soil--a matter of importance in predicting soil behavior. The K^+ ion is not considered chiefly because the amount of K^+ in the soil solution is seldom appreciable even where much exchangeable K^+ exists. Although the SAR concept is not fully accepted by all soil scientists, there is no doubt that the SAR of a soil solution in equilibrium with the exchange complex has a tremendous practical value. It is readily obtained, is sufficiently accurate for diagnosis and its interpretation in terms for the probable status of exchangeable Na^+ in the soil is relatively simple. The interpretation of soil behavior in terms of crop response is too complex for treatment here; interested readers should consut references 6, 8, 14, 17, 18, 19 and 22.

For determining the suitability of water for irrigation purposes, the SSP and the SAR are augmented by a third concept, that of "residual sodium carbonate" (RSC). The SSP and the SAR of an irrigation water are readily computed from the water analysis but their interpretation is complicated because irrigation water is altered as it becomes soil solution. If the irrigation water became soil solution simply by loss of water through evapotranspiration, no problem would exist. Unfortunately exchange reactions between cations occur, plants take out some constituents and add others (HCO_3^-) and precipitates such as $CaCO_3$ and $CaSO_4$

[illegible] solubilities are exceeded. The RSC concept, first pro-[illegible]ation (10,11) helps interpret some of these changes. [illegible] solutions containing Ca^{++} and HCO_3^- are concentrated by [illegible]ation, $CaCO_3$ often precipitates.

$$Ca^{++} + HCO_3^- \quad CaCO_3 + H^+$$

The equation indicates that alkalinity would favor precipitation of $CaCO_3$. Many irrigated soils in the Western States are alkaline, and some are strongly so. The consequences of such a selective precipitation in a solution containing mixed salts are important; it should be obvious that removal of Ca^{++} from solution will alter the Na^+/Ca^{++} ratio in solution. As the Na^+/Ca^{++} ratio (shown either as the SSP or the SAR) increases, the Sodium hazard increases. The RSC concept is a measure of potential sodium effect of precipitation of Ca and Mg carbonates.

$$RSC = (CO_3^- + HCO_3^-) - (Ca^{++} + Mg^{++}) \text{ in meq./l.}$$

When the RSC is positive, it would be possible to have a solution virtually free of Ca^{++}. In point of fact, one actually finds little or no Ca^{++} in soil solutions expressed from sodic soils of pH 8.5 - 10 where appreciable CO_3^- + HCO_3^- exist.

The extent to which the precipitation of Ca^{++} + Mg^{++} by CO_3^- actually occurs is still an unsolved problem. The irrigation [illegible], evapotranspiration loss, drainage characteristics of the

soil and many other factors all bear of this question. The RSC is, nonetheless, a valuable aid in interpreting the hazards to the use of a given irrigation water.

Figure 2 presents the system of interpreting quality of irrigation water based on Salinity Hazard and Sodium Hazard proposed by Wilcox (22,23). Wilcox (23) considers RSC as another criterion. Doneen (9) has proposed the use of "effective salinity," a scheme by which total salinity is reduced by the amounts of Ca^{++} and Mg^{++} which may be precipitated as $CaCO_3$, $MgCO_3$ or $CaSO_4$. Other classification schemes for irrigation waters have been proposed which include waters far more concentrated ($>$ 5000 micromhos/cm.) than those considered by Wilcox (Fig. 2). The requirements for handling more concentrated waters are discussed by Wilcox (23).

Specific Ion Effects

Effects of ions which are not attributable osmotic effects on water availability are described, for convenience, as toxic effects of the ion or salt in question. Under this definition toxicity may be either a direct effect of the ion on some process or an indirect effect on uptake or metabolism of essential elements.

The effects of high concentrations of specific ions or salts on plant growth is too complicated to present here. Bernstein

and Hayward (5,12) have reviewed the literature on this subject in some detail. Wide differences in plant response to a given ion or condition is the rule. The brief review which follows is chiefly to illustrate the range of toxicities encountered.

Specific ion effects are broadly of two types: (1) nutrient deficiencies caused by interference with the uptake process and (2) ion accumulations in injurious amounts.

The effects of high HCO_3^- and high Na^+ are examples of nutrient deficiencies. Continued use of high HCO_3^- waters (> 200 p.p.m.) has consistently caused chlorosis in deciduous fruits in the Pacific Northwest (16). The chlorosis can be cured by iron injection or by substituting water of lower HCO_3^- content. These same waters may be used with no adverse effects on most field crops. On sodic soils, deficiencies of Ca^{++}, Zn^{++} and Fe^{++} have been observed.

Injurious effects are more common. Toxicity of Na^+ has been noted for a few select plants; almonds, and avocado are injured by Na^+ at levels so low as to be regarded as negligible for most crops. Some plants (peaches and other stone fruits, pecans, avocados, grapes and citrus) are sensitive to Cl^- at levels which do not affect most truck and field crops. The case of B is probably worked out in the greatest detail. Many data are

available on the differences in B requirements among plants, their tolerance for excess B and on diagnosis of deficiencies and excesses. The concentrations of B necessary for the growth of plants of high B requirement may actually be toxic for plants very sensitive to B. Wilcox (23) lists the permissible level of B in irrigation waters according to crop sensitivity and the relative B tolerance of many crop plants.

Injuries to plants from excess As, Cu, Zn, Mo, and Mn are known. The aquatic environment, i.e., the soil solution, is not the point of diagnosis, however. It is simple enough to demonstrate injury to plants grown in sand or solution culture from Cu, Zn, etc., (21) but the data derived from such experiments are essentially meaningless when it comes to diagnosis of toxicities under field conditions. The solid phase of the soil, mineral and organic, absorbs most of these elements so strongly that their concentrations in the soil solution is often negligible and well below toxic limits set in solution or sand culture. Attempts to correlate plant uptake of such elements with soil supplies (8) usually use salt solutions, dilute acids, or chelating agents as extractants. There presently exists no reliable diagnostic tool for predicting injury to plants from these elements. Much remains to be done to develop sound diagnostic techniques for assessing deficiencies and

excesses of many elements.

Injuries to plants from F, Pb or Se arising from soil sources are rare or unknown. Plants do absorb these elements from soils but do not appear to be injured thereby. Fluoride injury to plants appears to be due primarily to atmospheric pollution. Animals, however, may be injured by consuming forages and waters containing large amounts of F, Pb, Se, and NO_3^-.

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DISCUSSION

STATEMENT: I presume that there is some question in the mind of some here as to why this type of discussion was included in this agenda. The quality of water for agricultural use is just as much a water pollution problem as any other use discussed today. One of the outstanding differences between this discussion of the agricultural phases of the water toxicity problem and the previous biological discussions, is that Dr. Moodie talked with much more assurance regarding the effect of toxic elements on plant. There is considerable in common between land and water plants and land and water environment, if these areas of common interest can be found. We in water pollution could learn considerable from the agricultural and soil scientists and much of the knowledge they have could be applied to some degree to our water quality problems. Actually, we are dealing with the water quality in our studies of soils because water is involved, although it may be soil water. There is one advantage that the agriculturists have over the fisheries biologist and that is

that his crop is always there when he wishes to study it, while fish are transient and may be many miles away.

Q. How common are the damages to which Dr. Moodie alluded in his talk?

A. In regard to the number of acres effected by excess concentrations of salt, there are thousands of such acres in the State of Washington. This is one of the hazards of irrigation development. There have been as many acres of land ruined by excess salt accumulation, due to a lack of understanding, as there have been acres developed for irrigation. This is a problem associated with improper drainage. It occurs under natural conditions in poorly drained soils and it occurs during irrigation when adequate drainage is not provided.

Q. In view of the problems arising from the use of water for irrigation and in view of the present surplus of food, shouldn't there be some restriction on the use of water for this purpose?

A. This is a question which always arises; however, the facts are that surpluses exist only for certain crops and not for others. As our population expands, it is estimated that by 1975 these surpluses will largely disappear. In addition,

the present food surplus is one of the most important national resource that we have against disaster. If we divert this water from irrigation to other purposes, we will be in a very difficult position when these surpluses disappear and we need this food for our expanding population. While agriculture is an industry, we have to remember that the individual operator in this industry is the farmer. He has no spokesman and as an individual operator must exist on his wages. You cannot simply divert water from this man as you can where you can juggle it from one use to another in order to find a better use for it.

Q. Did you say the farmer was an individual without a spokesman?

A. He doesn't have a spokesman in the area we are discussing. The farmer does not understand the implications of such things as the use of insecticides and pesticides as a part of a calculated risk to national health. Usually he does not know what you are talking about any more than the operator in an industrial plant knows about the consequences of the material he uses. He takes a calculated risk every year of his life--whether he's going to make a living or not. Agricultural production is in the hands of individuals who have to make a living.

Agricultural scientists know fairly well what the quality of water required for agricultural purposes must be. But they know far less about what agriculture does to the quality of water as it affects other people. There is a potential among the agricultural scientists to find this out, but they have no directive nor money to do this. I suggest that it would advance this problem of water pollution a great deal if use were made of the agricultural scientists on some of these problems. Some of their programs can be directed toward this area if there is support for it.

PARALYTIC SHELLFISH POISONING

John C. Girard*

Paralytic shellfish poisoning results from the ingestion of any of various species of molluscs that have significant amounts of a naturally occurring toxin. McFarren, et al., (1957) enumerate over 600 cases of such poisoning with 69 deaths throughout the world from 1793 to 1954.

The first recorded cases and deaths attributed to the poisoning on this continent were reported by Captain Vancouver in his journal published in 1798. In 1793, while on Vancouver Island, British Columbia, four members of his crew, one of whom died, became ill from eating roasted mussels. The fact that Vancouver wrote that his crew had some idea of treatment of those stricken indicates strong possibility that these were not the first cases of the illness. Since that time cases of the poisoning have been reported in California, Oregon, Washington, British Columbia, and Alaska on this coast and Nova Scotia, New Brunswick, Quebec, and Maine on the Atlantic Coast. The most recent outbreak on the Pacific Coast of any magnitude occurred in British Columbia in October, 1957, when 50 cases of

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the illness resulted from the consumption of toxic Pacific oysters (Davies, et al., 1958).

It is difficult to conclude that from the incidence of poisoning, this can be considered to be a major public health problem. Nevertheless, the fact that man can receive a fatal dose of the toxin from a single serving of highly toxic shellfish and that there is no known antidote requires our strict attention. At the present time, there are no means for predicting the occurrence of the toxin within shellfish. Prevention of the illness is based on a control program which identifies toxic shellfish before they reach the consumer. This requires submitting lots of shellfish from suspected areas to a laboratory assay procedure to ascertain the level of toxin within the shellfish.

McFarren, et al., (1960) writes "The first symptoms of poisoning (Meyer, et al., 1928 and Medcof, et al., 1947), in humans are associated with peripheral paralysis which may progress from a slight tingling and numbness about the lips to a complete loss of strength in the muscles of the extremities and neck and even to death by respiratory failure. In a moderately severe case, the tingling, stinging sensation around the lips, gums, and tongue develops in about five to thirty minutes after

consumption of the mussels. This is regularly followed by a numbness or a prickly feeling in the fingers and toes and, within 4 to 6 hours, the same sensations may progress to the arms, legs, and neck, so that the voluntary movements, as for example, raising the head, can be made only with great difficulty."

In reviewing the writings of the early workers on this problem, it is interesting to observe that they concluded that the cause of the poisoning was due to something the shellfish ingest. Probably the main difficulty confronting them was the unpredictable occurrence of the toxin in the shellfish. The fact that human cases appeared after eating shellfish, shellfish, became toxic very suddenly and evidently became safe to eat shortly thereafter, required investigation to be accomplished during an outbreak. Beginning in 1927 and continuing into 1937, Sommer and Meyer were able to ascertain the cause of toxic shellfish in California (Sommer, et al., 1937). These workers established that the toxin in the mussel Mytilus californianus was associated with the occurrence of the dinoflagellate Gonyaulax catenella and that these organisms were poisonous. They further established that the mussels were toxic only when G. catenella was in the overlying water and that the toxicity of the mussel was proportional to the number of G. catenella in the

water and in the stomach of the mussels. Sommer also fed cultures of G. catenella to mussels, thus making them toxic and after removing them from the organisms and placing them in sea water free from the organism, found the mussels to be free of toxin within a short time.

In studying the shellfish poisoning problem in the Bay of Fundy on the East Coast, Needler (1949) showed the causative organism to be Gonyaulax tamarensis. At the present time, the causative organism for the occurrence of paralytic shellfish poison in waters of the State of Washington is not known. In 1960, Dr. Sparks and associates initiated a study on the shellfish toxicity problem in Washington with one of the objectives being to attempt to ascertain the causative organism (Sparks, et al., 1961).

The present method used for determining the toxin in shellfish is a modification of one described by Sommer and Meyer in 1937. The collected meat of a representative lot of shellfish is washed, allowed to drain and then ground until a homogeneous mixture is obtained. One hundred grams of the material is introduced into a beaker into which is added 100 ml of 0.1 N hydrochloric acid. The mixture is stirred thoroughly, heated to allow gentle boiling for 5 minutes, and allowed to cool to room temperature. The pH of the mixture is tested and if necessary,

adjusted to 3.0. The volume is then brought back to 200 ml and an adequate portion of the supernatant liquid is decanted off for the test. One ml portions are introduced intraperitonaeally into 3 mice weighing between 18 and 22 grams each and the median death time for the mice is determined. If the death time is less than 5 minutes, dilutions are made to obtain death time between 5 and 7 minutes. Applying the death time into a table and formula developed by Sommers, the amount of toxin in the extract is expressed in numbers of mouse units per 100 gram of meat. By definition a mouse unit is the minimum amount of poison required to kill a 20 gram mouse in 15 minutes when 1 ml of solution is injected intraperitonaeally.

The most important of early studies on the chemistry of the toxin was published by Sommer, et al., (1948). It was determined that detoxification occurs with increases in pH and temperature. At pH 5.0 there is a loss in toxicity of only 6% in 6 days at 25°C., but at a pH of 6.6 this loss increases to 35% and 74% at a pH of 11.5. Likewise, an increase in the temperature from 25° to 100° at 5.0 causes the loss in toxicity to increase from 6% in 6 days to 85% in one day. Recently Shantz, et al., (1957) concluded that both the clam and mussel toxin must be the same or nearly the same with a molecular formula of $C_{10}H_{17}O_4N_7.2HCL$.

Beginning in 1944, personnel from Northwestern University, University of California, and the Chemical Corps Biological Laboratories at Fort Detrick were successful in the purification of the toxins from the California mussel M. californianus and butter clams Saxidomus giganteus (Shantz 1960). It was found that the toxins are among the most potent known to man and that the purified toxin from mussels is the main cause of "mussel poisoning" described by Meyers, et al., (1928). An interesting aspect of the work on the butter clam is that in some cases it was required to process up to 8 tons of the clams to obtain one gram of the toxin.

For many years various workers recognized some of the inadequacies of the use of the mouse unit as a means of expressing the amount of the toxin present in shellfish. This is primarily related to the varying tolerance of the toxin by different strains of mice. In 1955, research was initiated to implement the use of the purified toxin in the standardization of animal assay. This was successful and presently, by using the CF value for the mouse colony used, the amount of toxin is expressed by micrograms of toxin per 100 gram of meat.

At the present time, work is being done in attempting to develop a chemical method for the quantitative determination of the toxin in shellfish. This test may be of particular value in

areas where mice are difficult to obtain, but its usefulness must be further studied (McFarren, et al., 1960).

The phytoplankton of concern possess the ability to reproduce themselves into huge populations in a very short time, thus causing shellfish feeding on them to become toxic. Although each species of shellfish appear to concentrate the toxin at a rate different from other species, all bivalves should be considered suspect in critical areas during periods when the causative organism is present. Of particular interest is the fact that the butter clam evidently may retain the toxin for long periods of time, thus complicating this aspect of the problem.

On this coast, all reported cases have come from the consumption of mussels, butter clams and Pacific oysters. In California and Oregon, seasonal closure of coastline areas is evidently the primary means for providing the necessary protection. In Alaska, high levels of toxin in butter clams occurring throughout the year prohibit the commercial exploitation of vast shellfishery resources. In Washington, a monitoring program is provided, in addition to excluding shellfishery utilization in certain areas, between April 1, and October 31. As in other jurisdictions, shellfish areas are closed when the amount of the toxin in shellfish exceeds 80 micrograms per 100 ml of the meat.

Regarding the question of the minimal amounts of the toxin required to produce intoxication in humans, Medcof, et al. (1947) concluded that there is wide variation of severity of reaction to nearly equal doses amongst the people involved and that some people have a natural tolerance to the toxin.

For many years, it was believed that fatality to humans would occur only upon ingestion of approximately 40,000 mouse units. At the present time, it is generally believed that the minimal lethal dose may be far below this level.

Probably the most perplexing facet of the toxicity problem has been the attempt to correlate shellfish toxicity with such ecological factors as meteorological and oceanographic information. It appears that on this coast, the highest shellfish toxicities occur in the summer. There are indications that along the coast there are upwellings of colder water producing water temperatures 3 or 4 degrees lower than the average to be expected for the latitude. The average summer water temperature in areas on this coast when toxicity appears ranges from 10° to 14°C. (McFarren, et al., 1960). In studies of the Bay of Fundy, Needler (1949) has been able to show that the organism G. tamarensis may appear after the surface water reaches a temperature of 10°C. This information has proven to be quite useful to the

control authorities in their monitoring program for that area.

Our present understanding of the toxicity problem, as it occurs in Washington, is essentially limited to knowledge of levels attained in various species of shellfish, frequency of occurrence, and areas associated with the toxin. Unfortunately, little has been done in studies relating to the identification of the causative organisms, the ecological conditions which govern the incidence of toxin, or the means for predicting the occurrence of toxin in shellfish. Sparks, et al. (1961) have recently released a status report on their attempts in gaining information on some of these aspects of the problem and we are looking forward to further developments.

In closing, may I state that these discussions are not to be considered a complete delineation of the problem, but only to provide some understanding of the basic elements of the problem as it exists in this area.

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DISCUSSION

Q. Does cooking effect the toxicity of shellfish toxin?

A. The average cooking time will destroy between 35 and 60% of the toxic properties depending upon the method of cooking.

Q. How are you monitoring the oyster industry in Washington as regards shellfish toxin?

A. We are running samples from a number of areas every two weeks.

Q. If you find the toxin present, will this be in time to stop the shipment of the oysters to the market?

A. We are not testing actual shipments but are making our tests in the actual growing areas and hope to close these areas before the toxin reaches a critical level. Of course, there are other types of shellfish involved such as molluscs and clams. Those areas must also be controlled. Actually, most of the oysters are grown in areas outside of those where the toxin has been found.

Q. Isn't this problem usually associated with the outer waters of the sea, rather than those of bays and estuaries?

A. Yes.

Q. In what part of the shellfish is the toxin usually found?

A. Largely in the digestive tract of the oyster. In the butter

clam about 60% of toxin is in the siphon of the clam. I think this is the only species studied where it is outside of the digestive tract.

TOXICITY BIOASSAYS VERSUS CHEMICAL ANALYTICAL METHODS - THEIR ADVANTAGES AND LIMITATIONS

Peter Doudoroff*

Toxicity bioassays of industrial wastes are necessary, in addition to chemical analyses, for the following reasons:

1. The substances that are toxic to aquatic life and that can occur as important components of complex industrial effluents are very numerous. It is often impossible to predict just which toxic components will be present and important in a particular effluent, and so need to be tested for or measured chemically. Indeed, some of the most important toxic constituents of common wastes, such as pulp mill effluents, have yet to be chemically identified.

2. No chemical analytical methods have as yet been devised for the rapid determination of low yet dangerous concentrations of many toxic substances in waste waters. Those analytical methods that have been developed for the routine examination of polluted waters often fail to distinguish between highly toxic

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constituents and other chemically related components that are relatively or entirely harmless.

3. The toxicity of the many toxic water pollutants to various aquatic organisms, in different natural waters, and in various possible combinations, has not yet been adequately investigated. Therefore, the toxicity of a complex waste to the important organisms present in the receiving water often cannot be reliably predicted even when all of the waste components have been identified and accurately measured chemically. It must be determined empirically by biological test.

Routine toxicity bioassays of industrial effluents cannot, however, provide all of the information concerning the properties of these effluents that may be required in connection with a toxicity investigation. Some of the reasons why chemical analyses may be essential in addition to such routine toxicity bioassays follow:

1. In order to devise appropriate corrective measures, it may be necessary to know whether the observed toxicity of a waste or receiving water is or is not ascribable to some particular chemical component known or believed to be present. Routine bioassays demonstrate only the toxicity and not the cause thereof, although the appearance and behavior of the test animals may

suggest the nature of the principal toxicant involved. Only chemical analysis can definitely establish the presence of a particular toxicant in concentration sufficient for producing the observed toxic effects on the test animals.

2. Relatively short-term, routine, acute toxicity bioassays are capable of demonstrating only the presence of rapidly lethal or obviously harmful concentrations of toxicants. They are unsuitable for detecting in polluted waters low concentrations of toxicants that can produce only chronic effects, such as long delayed mortality and sublethal impairment of functions or performance of organisms. Only sensitive chemical tests and relatively difficult and prolonged biological tests may be capable of revealing the presence of these chronically harmful concentrations of toxicants.

3. Even acutely toxic concentrations of pollutants in waste waters may be demonstrable by bioassays of several days' duration only. Continual or frequent renewal of the test media in the course of the bioassays may be necessary, because many toxic pollutants do not persist long in dilute solutions, being subject to bacterial decomposition, chemical oxidation, etc. Only chemical analytical methods thus may be appropriate for rapid and easy detection even of acutely toxic concentrations of some

pollutants in waste waters, which may be essential to proper control of waste discharges.

4. Some waste constituents that are not themselves demonstrably toxic to aquatic life are nevertheless potentially harmful, being potential sources of highly toxic substances. For example, complex metallocyanide ions are not markedly injurious to fish, but hydrocyanic acid formed through their dissociation or decomposition (e.g., when the hydrogen-ion concentration is increased, or under the influence of light) is extremely toxic. Thus, ordinary bioassays may not reveal the latent acute toxicity of certain wastes whose dangerous though nontoxic components can be detected by appropriate chemical analytical methods.

It has been explained why neither toxicity bioassays nor chemical tests alone can be relied upon in seeking to evaluate the actual and potential toxicity to aquatic life of wastes and polluted waters and to regulate discharges of toxic wastes. Both of these complementary approaches to toxicity problems clearly are necessary. There is need, however, for further development and refinement of both of these approaches.

Attempts must be made to develop toxicity bioassay methods suitable for detecting as promptly as possible the presence of

slowly lethal and sublethal concentrations of toxicants in polluted waters. Low concentrations of toxicants that impair the appetite and growth of fish, interfere with their normal activity or swimming performance, or seriously alter their behavior patterns can be eventually as destructive to valuable fish populations as those that are rapidly fatal. The components of complex wastes likely to have these effects on aquatic life in receiving waters may not even be the same components as those that cause death at high concentrations of these wastes. The former components may still be present in harmful concentrations when the concentrations of the latter components have been reduced by dilution to harmless levels. Bioassay methods suitable for detecting injurious effects of wastes at concentrations far below those that cause rapid mortality of the test animals thus can be even more instructive than those now in common use.

One may not assume that any given measurable response of an organism to low concentrations of a toxicant is necessarily evidence of significant harm to that organism. Nor may one assume that a response observed at harmful concentrations of some toxicants will occur also at harmful concentrations of all other toxicants. Some of the responses, such as changes of breathing rate, can be transient or essentially adaptive. Only when the demands placed upon an organism by a change of its environment

overburden its capacity to adapt itself to such changes, so that its ability to survive, grow, and reproduce is impaired, can the change be said to be truly harmful. The proper selection of responses to be observed and measured in seeking to detect harmful sublethal concentrations of toxic pollutants by bioassay, and also the interpretation of the observations, thus are not simple matters.

Increased standardization of toxicity bioassay methods is a desirable objective, and continued efforts are being made in this direction. However, excessive or premature standardization can impair the usefulness of these methods and may tend to discourage experimentation which is essential to progress.

Chemical analytical methods also need much improvement. New methods especially suitable for application to toxicity problems must be developed. Unfortunately, most chemists working in the field of water pollution in the past have concerned themselves very little with the utility of their analytical methods in connection with problems in the toxicology of aquatic organisms. They have largely neglected the question whether a particular water pollutant being measured (because of its toxicological importance) is or is not actually present in a form that is toxic to aquatic life. For example, precise and sensitive

methods have been developed for the determination of cyanide in polluted waters without seeking to distinguish between cyanide present as free, molecular hydrocyanic acid and that present in the form of complex metallocyanide ions. Likewise, available analytical methods for the determination of various toxic heavy metals fail to distinguish between simple metallic cations and any metal present in the form of complex ions or colloidal precipitates (insoluble carbonates, hydroxides, etc.). Yet, it is well known that free, molecular hydrocyanic acid and most of the heavy metal cations are exceedingly toxic to fish, whereas complex metallocyanide ions and precipitated insoluble metallic compounds (including the cyanides) are relatively or entirely harmless. The available analytical methods, involving serious disturbance of ionic equilibria in water samples, can be useful to the biologist, but obviously they are not entirely satisfactory or adequate for the investigation and evaluation of the toxicity of polluted waters. Nevertheless, surprisingly little interest has been shown by water chemists in the past in the development of more appropriate analytical methods that would yield toxicologically more meaningful results. Some apparently find it difficult even to understand the need for such new methods.

Cooperative studies at Oregon State University recently have been directed toward filling the need for new analytical methods directly applicable to toxicity problems and for clear demonstration of the utility of such methods. Working with Professor Harry Freund of the Chemistry Department, C. R. Schneider has recently devised a highly satisfactory, as yet unpublished method for the determination of free, molecular hydrocyanic acid in complex cyanide solutions, without undue disturbance of ionic equilibria. This has made possible reliable prediction of the acute toxicity to fish due to cyanide of waters containing cyanide in combination with various heavy metals. It is to be hoped that other chemists will in the future take an increasing interest in similar problems. There is a clear need and opportunity for closer collaboration of chemists with biologists in the water pollution field.

BIOASSAY - THE BIVALVE LARVAE TOOL

Charles E. Woelke*

Problem:

Those of us working with bioassays sooner or later feel the need for better tools for measuring toxicity. A bioassay method which is economical to conduct, yields reproducible results, detects either chronic or acute toxicities or both, can be used either in the laboratory or field any season of the year, and utilizes a non-migratory commercially important organism would seem to approach the ideal situation. Work being conducted in a number of areas indicates that bioassays using bivalve larvae may fulfill these requirements. I am speaking primarily of the larvae of clams and oysters.

Materials and Methods:

The procuring of bivalve larvae is not difficult and a trained biologist in a marine laboratory can fairly quickly and easily learn the techniques. During the normal reproductive season, adult clams and oysters are available from their native habitats for spawning. Other months of the year these molluscs

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can be "conditioned" for spawning by placing them in flowing warm sea water for three to four weeks. Adult clams and oysters with ripe gonads are naturally spawned by increasing the water temperature 5-10° C. and adding sperm to the water. The fertilized eggs are added to water containing the material bioassayed, at a density of 15-20,000 per liter by some workers, less by others. After 48 hours at 20° C. the eggs normally develop to free swimming straight hinge larvae. At this time aliquot samples are taken and microscopically evaluated to determine the effect of the material on the embryonic development of the eggs. Bioassays may be terminated at this point, or the larvae fed on phytoplankton and grown in the variable. Often materials which have little or no effect on embryonic development at a given concentration will have marked effects on either the growth of the larvae or their ability to metamorphose from larvae to juveniles. This is particularly true of some of the insecticides and herbicides. These bioassays can be conducted in the laboratory with dilutions of materials you wish to test. Bioassays can also be conducted using water from the field in which the variable is known to occur.

Some workers have conducted shorter bioassays by observing the effects of a material on the swimming of the larvae over a

four hour interval and the subsequent recovery of swimming ability. Other workers have conducted longer term bioassays with young oyster spat which have attached to glass slides. In these studies it is possible to see the physiological response of the oyster, since the slide acts as a window through which internal organs of the oyster spat may be observed.

Technique Problems:

From this brief summary of the method one might assume there are no control or technique problems with the bivalve larvae bioassay. This, of course, is not true; the important point however is that most of these problems can be either solved or controlled without undue difficulty.

Among the problems associated with the larval bioassay, a major one is to procure larvae whenever desired. Earlier workers sacrificed adults with ripe gonads and washed the eggs free from the female. Due to the frequency with which unsatisfactory results were achieved from apparently immature eggs, this practice was dropped. Larvae are now procured only from naturally liberated eggs. This method has resulted in consistent reproducible results. The drawback to this procedure is the ever present danger of being unable to achieve a spawning when desired. It has been our experience that a stock of 30-40 thermally condi-

tioned adults is adequate to assure a spawning for bioassay purpose at any planned time plus or minus 30 minutes.

"Age of the water" used, i.e., time elapsed between removal from the natural environment and introduction of the eggs to the water, has an effect on the results. We have found that water held two hours or less in one gallon containers has no measurable effects on bioassay results. Water from 3 to 6 hours old may have an effect, water which is 24 hours old has very definite effect on the results of larval bioassays. In our work we make every effort to utilize water less than "3 hours old."

The "quality" of control water is also of concern, since metal ions, bacteria, temperature, plankton and other suspended material may affect results. To minimize the danger of problems from these sources we utilize water drawn through non-metallic, non-toxic lines with a hard rubber pump. This water is heated to a constant temperature of 20° C., filtered through a 5 micron filter and treated with ultraviolet light. While much of this seemingly elaborate treatment is not necessary at all times, we prefer to use control water of as near a constant quality as possible.

We have found that the size of containers used and the type (glass or plastic) have slight statistical effects on our results--

not great enough to be of concern but nevertheless great enough to indicate the merit of utilizing the same size and type of container in any given series of bioassays.

The age of developing embryos when introduced to the variable bioassayed must be controlled. With many materials response changes (generally declines) with increasing age, therefore, to achieve comparable results we introduce developing embryos to the material being bioassayed between one and two hours after fertilization. This practice is satisfactory where we are concerned with relative toxicities only. Where minimal toxicity levels are desired, fertilization must occur in the variable.

Where low level effects are being considered, problems of sampling variation can mask results; however, this problem can be minimized by increasing the number of cultures in the controls and variables to increase the statistical reliability. In our work a given bioassay will usually be made up of 60 cultures, with at least 10% of them controls and duplicate or triplicate cultures of each variable.

Our 48-hour bioassays are generally carried on in one liter polyethylene beakers seeded with 10-15,000 eggs; however, technique evaluation studies with densities up to 60,000 per liter have been conducted. At 60,000 per liter there is some indica-

tion of adverse effect from "over seeding."

The 48-hour bioassays are evaluated on the basis of percent abnormal larvae. Normal larvae are those which are fully shelled. Some of those called normal are often of irregular shape and undersized; however, using this "full shelled" criterion eliminates much of the variability which arises when several people are evaluating the larval samples. It has been our experience that those larvae termed abnormal will not feed and grow, and as a result die within a few days. A further basis for considering the abnormals undesirable is the absence of abnormal larvae in plankton samples from nature. We, therefore, use percent abnormal larvae as an index of effect and consider a material which induces abnormality in excess of the controls to be adversely effecting the larvae. Where over 50% of the larvae are abnormal it is very probable that the lethal threshold has been reached. Where abnormals reach 90% we consider the variable to be clearly lethal to the larvae.

Throughout the entire method every precaution is taken to ensure a healthy group of larvae for use in bioassays, however, occasional inferior larval lots are encountered. These are readily detected by the percent abnormality of the controls--we totally reject any bioassay where our controls have in excess of

10% abnormals; between 5-10% we are extremely cautious in making firm conclusions. We prefer to deal with larval groups where the controls average less than 2.5% abnormals.

In addition to the aforementioned problems, feeding rates and infestation of the cultures with other organisms--especially small marine worms and crustaceans--must be solved where growth, survival and setting of the larvae are studied. The problem of feeding the larvae in these longer studies materially increases the complexity and cost of a larval bioassay program. As a general rule, we do not attempt to feed and grow larvae unless we have reason to suspect effects which are not detected in the 48-hour bioassays.

Depending on the type of work being done, all of these problems, part of them, or additional ones may be encountered when using the bivalve larvae bioassay. It is sufficient to reiterate that while problems exist, they are not insurmountable.

Results from Larval Bioassays:

Among those who have been working with bivalve larvae as a bioassay tool in other areas are Dr. Loosanoff and his associates on the East Coast, and Okuba and Dr. Imai and his associates in Japan. In the Pacific Northwest, Willie Breese of the Oregon State Yaquina Bay Laboratory, John Denison of the Rayonier Research Laboratory, and the Washington State Department of Fisheries, have

done varying amounts of bioassay work utilizing bivalve larvae. While in many respects this bioassay tool is in early stages of development, the costs in terms of equipment, outlay and time required to achieve results make it an extremely attractive one, especially when dealing with concentrations of materials which appear to act in a chronic rather than acute manner on the adult organisms.

Thus far these workers have reported studies with industrial wastes, salinities, temperatures, insecticides, weedicides, chemicals, silt and effects of materials such as stainless steel, plastics, metals, etc., on water quality.

Toxicity information reported from larval bioassays is not extensive as yet; however, I will mention a few of the results I am aware of. In general, water which has been oftentimes only briefly in contact with metals, especially some types of stainless steel, is lethal. Many plastic materials, notably pure polyethylene, are non-toxic; many of those which contain plasticizers are lethal. Glass is non-toxic. Potassium cyanide is lethal at 0.014 p.p.m., mercuric chloride at 0.027 p.p.m., copper sulphate at 0.04 p.p.m., sodium sulfide at 2.44 p.p.m., sulphuric acid at 33.11 p.p.m., Roccal at 1 p.p.m., Dowicide A about 10 p.p.m., Dowicide G at 0.25 p.p.m., NABAM at 0.50 p.p.m.,

allyl alcohol at 2.5 p.p.m., DIURON at 5 p.p.m., NEBURON at 2.4 p.p.m., Sevin at 5 p.p.m., toxaphene at 10.0 p.p.m., Guthion at 1.0 p.p.m., Dicopthion at about 2 p.p.m., and one of our big local problems, S.W.L., has been found lethal at 13 p.p.m. when the eggs are fertilized in the S.W.L. It cannot be stated that all of these values were derived under exactly the same procedures outlined in this report; however, the general techniques would appear to be the same, and I would expect the same general parameters would be reached by any of the workers mentioned. In general, where differences of results have been encountered, these differences have been due to either technique or interpretation differences.

In Operation:

At our Point Whitney Laboratory we routinely use 48-hour development of Pacific oyster larvae as a bioassay tool. New materials such as plastics, paints, chemicals and even wood (treated and untreated), which will come in contact with our laboratory water supply are bioassayed before we use them. We also have been bioassaying the natural waters within 4 miles of our laboratory for nearly a year to collect data on the statistical validity of the method, its reproducibility, and the range of variation found in the larval response to "field water." This

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past summer we conducted extensive "field water bioassays" with marine water from a number of areas other than Hood Canal. At present, we are statistically analyzing these data.

Future Work:

We expect in the immediate future to conduct bioassays of water samples from the field as easily as we now make chemical analysis of these same samples. We plan extensive use of larval bioassays in areas where shellfish problems exist to ascertain whether adult mortalities, poor growth, reduced fatness or reproductive failures are related to poor water quality as measured by the larvae. Finally, we hope to be able to bioassay fractions of complex wastes where often only a few milliliters of extracted material is available for bioassay purposes.

Application and Interpretation:

One major stumbling block to the general acceptance of this bioassay tool by lay and technical people has been that it deals with only one phase of the life cycle of the organism. The contention often put forth is that while biologically interesting, the larval approach is not realistic, since it does not necessarily reflect the effect on the adult, which after all is the item of economic interest. I must object to this rather fuzzy thinking on the part of these persons and point out that if a

material breaks the life cycle of the organism considered, very soon there will be no adults to worry about. In my opinion any material which breaks the life cycle of an organism at the reproductive stage represents an adverse effect on the adult. A more valid objectiion, however, deals with lower concentrations of a material which have an adverse effect on the larvae but are not necessarily lethal. These critics maintain that we cannot validly infer "effect" on the larvae as evidence of long-range effect on the adults. This objection does have merit. Work is currently being carried on by several agencies which should provide data on the relationship of effect on larvae versus effect on the adult. In the interim, I prefer to take the stand that an adverse effect on the larvae most probably will be reflected in undue stress on the adult. Under stress the adult's normal resistance to disease, changes in ecology, changes in temperature, salinity, dissolved oxygen or other environmental variables may be adversely affected.

BIOASSAY STUDIES AT NANAIMO, BRITISH COLUMBIA

Don F. Alderdice*

When an investigator is faced with a practical problem in water quality control, the prevailing approach entails his attempt to provide an estimate, based on some suitable response metameter, representing the maximum alteration which may be permitted in terms of a particular additive or change. Moreover, the estimate is usually derived by consideration of the response of a particular species and age of fish selected because of its availability or association with the situation being examined. The dosage-response relationship is normally obtained where the response metameter is a function of the concentration or level of the variable in question, and of the time of exposure required for a sample of test animals to respond.

The question now arises: How representative is the estimate so provided of the dose-response relationship if the ancillary conditions under which the estimate was made are allowed to vary? In effect, variation in the environment is the rule, and estimates of effect in terms of a change in one variable in the

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environment, obtained under constrained experimental conditions, may not be expected to apply without error to combinations of ancillary variables at levels other than those under which the estimate was obtained.

The question of the possible variability of a response metameter, upon which practical estimates are based, is one that is being considered at Nanaimo. The basis for the studies lies in multivariate analysis. The experimental approach is based on the work of Box (e.g., Biometrics, 10(1):16-60, 1954). An example of the method is outlined for the response of coho salmon smolts, in terms of median resistance time, to 3 mg/L of sodium pentachlorophenate over levels of salinity, temperature and dissolved oxygen as ancillary variables.

The response of coho smolts was examined over the combinations of a composite factorial design for estimation of the slope coefficients in the polynomial

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

where

Y = response time, minutes

x_1 = f (salinity)

x_2 = f (temperature)

x_3 = f (dissolved oxygen)

Analysis provided

$$Y = 51.0867 + 9.0469x_1 - 10.2603x_2 - 0.0068x_3 + 5.4581x_1^2 - 1.9932x_2^2 - 2.6706\ x_3^2 - 2.5000x_1x_2 + 2.5000x_1x_3 + 0.2500x_2x_3$$

Reduction of the polynomial to its canonical form, involving the transformation of axes to a set conforming to the geometric configuration of the response domain, provides an equation capable of more straightforward interpretation. Thus

$$Y - Y_s = 5.8356X_1^2 - 2.0648X_2^2 - 2.9766X_3^2$$

where Y_s = the response time at the centre of the domain and the canonical variables

$$X_1 = 0.9779(x_1 - x_{1s}) - 0.1539(x_2 - x_{2s}) + 0.1418(x_3 - x_{3s})$$

$$X_2 = 0.0907(x_1 - x_{1s}) + 0.9221(x_2 - x_{2s}) + 0.3763(x_3 - x_{3s})$$

$$X_3 = 0.1883(x_1 - x_{1s}) - 0.3557(x_2 - x_{2s}) + 0.9155(x_3 - x_{3s})$$

where x_{is} = the value of x at the domain centre.

In this particular case the response domain is of elliptical hyperboloid configuration with the optimum response time remote from the domain centre along th X_1 axis.

Having defined the 3-factor domain, a further series of tests were conducted along the X_1 axis to find the response optimum.

Results of this study are as follows: Over all levels of salinity between 0-27‰ S, temperatures between 0-14° C, and dissolved oxygen levels between 3 and 8 mg/L, the unique combination of these factors providing maximum resistance time to 3 mg/L, the unique combination of these factors providing maximum resistance time to 3 mg/L of sodium pentachlorophenate was at

15‰ S

2.57° C

5.31 mgO_2/L

The geometric centre of the response domain was at

0.50‰ S

4.28° C

4.27 mgO_2/L

Over the levels of the three ancillary variables considered, the median resistance time corresponding to the set of levels providing the smallest resistance time was approximately one-half of that provided by the set of levels providing the greatest resistance time. Variations in response time were such that maximum tolerance was provided at a unique locus approximately at 15‰ S, 2.57°C and 5.31 mgO_2/L.

Interpretation of the results may be premature at this time. One interesting consideration deserves some comment: The

oxygen level at the response optimum. Observation indicated that the velocity of the response was activity-dependent. At high oxygen levels the test fish were considerably more active than those tested at low oxygen levels. However, below about 5 mgO_2/L the effect of reduced oxygen concentration appears to influence resistance in spite of reduced activity in the low-oxygen tests. It appears that a compromise is reached in this instance between hypoxial and activity-induced stresses at about 5 mgO_2/L.

The method employed in this study is capable of quite general application to n quantitative variables. The experimental designs employed are standard or composite complete or fractional factorials. Although there are rigorous computations associated with the method, the amount of experimental time involved in the use of composite designs is considerably less than that required for complete factorial designs.

The method of multivariate analysis outlined is considered to be a useful technique in considerations of the manner in which the response of an animal may be altered under the variations of environmental conditions to which, at one time or another, such an animal may be subjected.

(MS in preparation for submission to Journal of Fisheries Research Board of Canada, 1961.)

DISCUSSION

STATEMENT: A book which covers fairly well the material presented in the previous paper is that of Turnbull and Eakin, entitled "Introduction to the Theory of Canonical and Matrices," published by Dover Publications, Inc., New York.

Q. How many individual bioassays were required to come out with those three numbers (salinity, temperature, and dissolved oxygen)?

A. Thirty.

Q. This seems to be considerably fewer than would be necessary if you were to hold two of these items constant and vary the third. That is, you could run experiments with constant salinity and temperature and vary the oxygen.

A. You could do this, but this will not satisfy the situation that we set up in the first place. If you are considering that certain constants are zero, this might apply. This is not the case, however, since all of these items vary. The point is, therefore, you cannot make the assumption that these variables are zero.

ATTENDANCE AT THE TENTH SYMPOSIUM

November 14, 1961

James L. Agee	U. S. Public Health Service	Portland
Don F. Alderdice	Fisheries Research Board of Canada	Nanaimo, B.C.
Ferman R. Amberg	Crown Zellerbach Corporation	Camas
Aven M. Andersen	Washington Dept. of Fisheries	Brinnon
E. R. Anthony	U. S. Geological Survey	Portland
Leo L. Baton	Oregon State Board of Health	Portland
William J. Beck	Shellfish Sanitation Laboratory	Gig Harbor
Donald J. Benson	Oregon State Sanitary Authority	Portland
Thomas P. Blair	Oregon State Board of Health	Portland
R. O. Blosser	Nat'l Council for Stream Imprvm't	Corvallis
W. P. Breese	Oregon State University Fish Lab.	Newport
James E. Britton	U. S. Public Health Service	Portland
Charles T. Bryant	U. S. Geological Survey	Portland
William E. Bullard	U. S. Public Health Service	Portland
John V. Byrne	Oregon State University	Corvallis
Richard J. Callaway	U. S. Public Health Service	Portland
Andrew Carey	Oregon State University	Corvallis
Dale A. Carlson	University of Washington	Seattle
Henry P. Carsner	Northwest Weed Service	Tacoma
George G. Chadwick	U. S. Public Health Service	Corvallis
David B. Charlton	Charlton Laboratories	Portland
R. M. Chatters	Washington State University	Pullman
William D. Clothier	Oregon State Fish Commission	Portland
J. F. Cormack	Crown Zellerbach Corporation	Camas
R. E. Dimick	Oregon State University	Corvallis
Peter Doudoroff	U. S. Public Health Service	Corvallis
Donald P. Dubois	U. S. Public Health Service	Portland
Gilbert H. Dunstan	Washington State University	Pullman
Leonard B. Dworsky	U. S. Public Health Service	Portland
Edward F. Eldridge	U. S. Public Health Service	Portland
Dorothy McKey Fender	Portland State College	Portland
Harry Freund	Oregon State University	Corvallis
Herbert F. Frolander	Oregon State University	Corvallis
John Girard	Washington State Health Department	Seattle
Dolores Gregory	Portland State College	Portland
Robert A. Gresbrink	Oregon State Board of Health	Portland
George H. Hansen	Washington Pollution Control Comm.	Yakima
Eugene P. Haydu	Weyerhauser Timber Company	Longview
D. K. Hilliard	U. S. Public Health Service	Anchorage

Dale A. Hoffman	U. S. Public Health Service	Portland
Ralph H. Holtje	U. S. Public Health Service	Wash., D.C.
G. LaMar Hubbs	U. S. Public Health Service	Anchorage
Gary W. Isaac	Rayonier, Incorporated	Shelton
Bryan Johnson	Washington Pollution Control Comm.	Olympia
C. R. Johnson	Portland State College	Portland
D. C. Joseph	California Fish & Game Department	Sacramento
Max Katz	University of Washington	Seattle
Stanley Knox	Washington Pollution Control Comm.	Olympia
L. B. Laird	U. S. Geological Survey	Portland
Robert E. Leaver	Washington State Health Department	Seattle
Bob Lewis	California Fish & Game Department	Redding
Alfred Livingston	Washington Pollution Control Comm.	Olympia
Donald Lollock	Oregon State University	Corvallis
Jim McCarty	University of California	Berkeley
James E. McCauley	Oregon State University	Corvallis
R. K. McCormick	Washington State Health Department	Seattle
Robert McHugh	Oregon State Board of Health	Portland
E. A. McKey		
James A. Macnab	Portland State College	Portland
Robert J. Madison	U. S. Geological Survey	Portland
Nick Malueg	U. S. Public Health Service	Portland
Harold E. Milliken	Oregon State Board of Health	Portland
Al Mills	Washington Pollution Control Comm.	Olympia
C. D. Moodie	Washington State University	Pullman
W. Allan Moore	U. S. Public Health Service	Portland
H. P. Nicholson	U. S. Public Health Service	Atlanta
Phillip A. Olson	General Electric Company	Richland
G. T. Orlob	University of California	Berkeley
Eben L. Owens	Nat'l Council for Stream Imprvm't	Corvallis
Kilho Park	Oregon State University	Corvallis
Maynard W. Presnell	Shellfish Sanitation Laboratory	Gig Harbor
Edison L. Quan	Oregon State Sanitary Authority	Portland
Robert L. Rulifsen	Oregon State Fish Commission	Portland
J. F. Santos	U. S. Geological Survey	Portland
Carl R. Schneider	Oregon State University	Corvallis
William B. Schreeder	U. S. Public Health Service	San Francisco
Robert W. Seabloom	University of Washington	Seattle
Dean L. Shumway	Oregon State University	Corvallis
Albert K. Sparks	University of Washington	Seattle
J. D. Stoner	U. S. Geological Survey	Portland
John C. Stoner	Lane County Health Department	Eugene
Philip N. Storrs	U. S. Public Health Service	San Francisco
Arporna Sribhibhadh	University of Washington	Seattle

Clarence M. Tarzwell	U. S. Public Health Service	Cincinnati
John D. Thorpe	Lane County Health Department	Eugene
Roger Tollefson	Consulting Biologist	Toledo
W. W. Towne	U. S. Public Health Service	Portland
R. A. Wagner	University of California	Berkeley
D. T. Walsh	Washington Department of Fisheries	Brinnon
Donald G. Watson	General Electric Company	Richland
E. J. Weathersbee	Oregon State Board of Health	Portland
Bill Webb	Idaho Department of Fish & Game	Boise
Donald R. Well	Washington Department of Fisheries	Brinnon
Henry O. Wendler	Washington Department of Fisheries	Vancouver
W. Q. Wick	OSU Extension Service	Tillamook
Don Wilson	Washington Pollution Control Comm.	Olympia
John N. Wilson	U. S. Public Health Service	Portland
Ruth Winchell	Portland State College	Portland
Charles E. Woelke	Washington Department of Fisheries	Brinnon
Charles D. Ziebell	Washington Pollution Control Comm.	Olympia